The Effect of Fluid Dairy Products on Satiety, Food Intake and Glycemic Regulation in Children

by

Shirley Vien

A thesis submitted in conformity with the requirements for the degree of Masters of Science
Graduate Department of Nutritional Sciences
University of Toronto

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2016

Abstract

The hypothesis that fluid dairy consumption before and with a meal decreases short-term appetite and food intake (FI) and improves glycemic control compared with a sugar-sweetened beverage in normal weight (NW) and overweight/obese (OW/OB) children was explored. Isocaloric (130 kcal) beverages of 2% milk, 1% chocolate milk, 1.5% yogurt drink, fruit punch and water were compared. In Experiment 1, FI was lower after chocolate milk and yogurt drink than water. In Experiment 2, milk led to lower pre-meal glucose and higher pre-meal GLP-1 than fruit punch in all children. When intake was adjusted for body weight (kcal/kg), post-meal glucose, insulin and GLP-1 increased, and ghrelin decreased more in OW/OB than NW children. Post-meal PYY was higher after milk in all children. Therefore, fluid dairy does not reduce FI and appetite more than other caloric beverages, but milk results in lower pre-meal glucose and more favourable responses in satiety hormones in children.
Acknowledgments

_I dedicate this thesis to my parents_

_Your unconditional love and support helped me to make this possible_

First, I want to express my gratitude towards my supervisor, Dr. Harvey Anderson for giving me the opportunity and making this research possible. I feel very privileged to have been able to study under your tutelage. I thank you for your knowledge and wisdom, and challenging me to become a better scientist.

I thank my Advisory Committee, Dr. Jill Hamilton and Dr. Nick Bellissimo for taking the time to provide guidance and contribute to my research. I thank my Examination Committee, Dr. Wendy Ward as the Appraiser and Dr. Thomas Wolever as Chair for making the examination process a smooth one.

_The Anderson Lab Family_

Shlomi Tamam, thank you for taking me on as a research volunteer and introducing me to the Anderson Lab. Without you, I wouldn’t have had the opportunity to be a part of a wonderful group. Dr. Bohdan Luhovyy, thank you for believing in me and giving me the opportunity to contribute to the lab as more than just a research assistant and encouraging me to pursue graduate studies. Dr. Dalia El Khoury, thank you for your guidance during my thesis work. Dr. Ruslan Kubant, thank you for your mentorship and friendship. You helped me gain confidence in my laboratory skills that I otherwise wouldn’t have. Drs. Barkha Patel, Shirin Panahi and Diana Sanchez Hernandez, my mentors and friends. I am forever grateful for all your encouragement and support. Thank you for all the time you’ve spent helping me before, during and after my thesis work, and for bringing positivity during stressful times. Thank you to Abraham Poon, Abigail Liu, Alex Schwartz, Chris Smith, Dr. Clara Cho, Emanuela Pannia, Maria Fernanda Nunez, Mukta Wad, Nazanin Barkhordari, Pedro Huot, Sascha Hunschede and Shari Berengut for being great colleagues to work with. You provided me with great conversations, contributed your ideas to my work and created great memories that I will take with me as I move forward. Lin Lin, thank you for being there during those long hours of work and your positive attitude throughout it all.
Thank you Ting Ting Liu, Chesarahmia Dojo Soeandy, Minja Simeunovic, Nikita Ambekar, Sofiya Porodko, Parneeta Singh, Jessica Chu and Melanie Yeung, the Research Assistants and Volunteers that helped me along the way. Your help was instrumental and I am grateful to all the early mornings and hours you put in! Thank you Munaza Jamil, Rachel Stegerand and the nurses at the Hospital for Sick Children, especially Cheryl Arneson for the weekend mornings and your good-natured attitude in helping us get through all the sessions.

Thank you to the parents and children who offered their time as participants in the study.

Thank you to my friends and family who have supported me throughout my academic journey and provided me with much needed times of good food and relaxation.

My sister, Hannah, thank you for the countless hours you spent helping me and beyond. Words can never express how thankful I am to have such a wonderful and selfless person in my life and especially one who is my sister and someone I can call my lifelong friend. One Unit until the end! My love, Alex Chung, thank you for your love, patience and never ending support in everything I do. Your confidence in me helped me pull through. Most of all, thank you for simply being you and being there when I needed you.

My Mom and Dad, thank you for your unconditional love and support, your patience and believing in me throughout this journey. I was able to succeed because of you. Thank you.
## Table of Contents

List of Tables ........................................................................................................................................ vii
List of Figures ....................................................................................................................................... viii
List of Abbreviations ............................................................................................................................ ix
List of Appendices .................................................................................................................................. x
Chapter 1 Introduction .......................................................................................................................... 1
Chapter 2 Literature Review ................................................................................................................ 2
  2.1 Introduction ......................................................................................................................................... 2
  2.2 Obesity and Type 2 Diabetes in Children and Adolescents ............................................................ 2
  2.3 Beverage Consumption by Children and Adolescents .................................................................... 4
    2.3.1 Sugar-Sweetened Beverage Consumption, Obesity and Type II Diabetes ............................ 4
    2.3.2 Milk Consumption, Obesity and Type II Diabetes ................................................................. 6
  2.4 Milk .................................................................................................................................................... 7
    2.4.1 Milk Proteins: Overview .................................................................................................................. 8
    2.4.2 Milk Fat: Overview ........................................................................................................................ 11
    2.4.3 Milk Carbohydrate: Overview ...................................................................................................... 12
    2.4.4 Milk and Milk Products ................................................................................................................ 13
  2.5 Regulation of Food Intake and Glycemia .......................................................................................... 15
    2.5.1 Physiology of Food Intake Regulation ........................................................................................ 15
    2.5.2 Hormonal Regulation of Satiety and Food Intake .................................................................... 15
    2.5.3 Regulation of Glycemia .............................................................................................................. 17
    2.5.4 Hormonal Regulation of Glycemia ............................................................................................ 18
  2.6 Milk in the Regulation of Satiety, Food Intake and Glycemia ......................................................... 20
    2.6.1 Satiety and Food Intake ................................................................................................................. 20
    2.6.2 Glycemic Control ........................................................................................................................ 21
  2.7 Summary and Research Rationale .................................................................................................. 22
Chapter 3 Hypothesis and Objectives .................................................................................................. 23
  3.1 Hypothesis .......................................................................................................................................... 23
  3.2 Objectives ......................................................................................................................................... 23
Chapter 4 Short-term effects of fluid dairy products on food intake and glycemic and
appetite hormone responses in children .............................................................................................. 24
List of Tables

TABLE 1. Energy and macronutrient composition of beverages and test meal .......................40
TABLE 2. Baseline characteristics of participants..................................................................41
TABLE 3. Experiment 2: Baseline blood parameters of participants ....................................42
TABLE 4. Effect of beverages on pizza intake, energy intake at the meal, cumulative energy intake and caloric compensation ..................................................................................43
TABLE 5. Experiment 2: Mean changes from baseline pre- and post-meal blood glucose and insulin, and appetite hormone responses unadjusted and adjusted for pre-meal beverage kcal/kg body weight ..........................................................................................................................44
List of Figures

FIGURE 1. Experiment 1: Subjective appetite changes from baseline pre- (0–60 min) and post-meal (60–145 min) of all, normal weight and overweight/obese children........................................45

FIGURE 2. Experiment 2: Subjective appetite changes from baseline pre- (0–60 min) and post-meal (60–145 min) of all, normal weight and overweight/obese children........................................46

FIGURE 3. Experiment 2: Serum blood glucose and insulin changes from baseline, adjusted for kcal/kg, pre- and post-meal .................................................................47

FIGURE 4. Experiment 2: Plasma GLP-1, PYY and ghrelin changes from baseline adjusted for pre-treatment intake (kcal/kg).............................................................................48
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
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<tr>
<td>BIA</td>
<td>Bioelectrical impedance analysis</td>
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<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CN</td>
<td>Casein</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>d</td>
<td>Day</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>FI</td>
<td>Food intake</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>HFCS</td>
<td>High-fructose corn syrup</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>Kcal</td>
<td>Kilocalories</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>NS</td>
<td>Not statistically significant</td>
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<tr>
<td>NW</td>
<td>Normal weight</td>
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<tr>
<td>OB</td>
<td>Obese</td>
</tr>
<tr>
<td>OW</td>
<td>Overweight</td>
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<tr>
<td>PYY</td>
<td>Peptide Tyrosine Tyrosine</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SSB</td>
<td>Sugar-sweetened beverage</td>
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<tr>
<td>T2D</td>
<td>Type II diabetes</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
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<tr>
<td>wk</td>
<td>Week</td>
</tr>
<tr>
<td>y</td>
<td>Year</td>
</tr>
</tbody>
</table>
List of Appendices

Appendix 1. Sample Size Calculation ................................................................. 75
Appendix 2. Experimental Protocol ...................................................................... 76
Appendix 3. Beverage & Pizza Composition .......................................................... 77
  7.3.1 Manufacturer Beverage Composition Data .............................................. 77
  7.3.2 Maxxam Analytics Beverage Composition Data ...................................... 77
  7.3.3 Pizza composition data from manufacturer ............................................ 78
Appendix 4. Recruitment Advertisements & Letters ............................................. 79
  7.4.1 Metro Newspaper Ad ............................................................................. 79
  7.4.2 The Hospital for Sick Children’s Research4kids Database Ad ............... 80
  7.4.3 The Hospital for Sick Children Advertising Poster ............................... 81
  7.4.4 Experiment 1: U of T Recruitment Letter ............................................ 82
  7.4.5 Experiment 2: U of T Recruitment Letter ............................................ 83
  7.4.6 Sick Kids Recruitment Letter ............................................................... 84
Appendix 5. Consent Forms ................................................................................... 85
  7.5.1 Experiment 1: Parent’s Consent Form ................................................... 85
  7.5.2 Experiment 1: Children’s Assent Form .................................................. 91
  7.5.3 Experiment 2: U of T Parent’s Consent Form ...................................... 92
  7.5.4 Experiment 2: U of T Child’s Assent Form .......................................... 98
  7.5.5 Experiment 2: Sick Kids Parent Consent Form ................................... 99
  7.5.6 Experiment 2: Sick Kids Participant Consent Form ......................... 105
  7.5.7 Experiment 2: Sick Kids Assent Form ................................................ 111
Appendix 6. Screening Questionnaires .................................................................. 113
  7.6.1 Telephone Screening Questionnaire .................................................... 113
  7.6.2 Background Information Questionnaire .......................................... 114
  7.6.3 Food Acceptability List Questionnaire ............................................. 115
  7.6.4 Puberty Questionnaire ........................................................................ 116
  7.6.5 Tanner Staging: Male .......................................................................... 117
  7.6.6 Tanner Staging: Female ........................................................................ 118
  7.6.7 Menstrual Cycle Questionnaire ......................................................... 120
  7.6.8 Dutch Eating Habits Questionnaire .................................................... 122
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.6.9</td>
<td>Physical Activity Questionnaire</td>
<td>124</td>
</tr>
<tr>
<td>7.6.10</td>
<td>Body Measurements</td>
<td>125</td>
</tr>
<tr>
<td>Appendix 7</td>
<td>Study Day Questionnaires</td>
<td>126</td>
</tr>
<tr>
<td>7.7.1</td>
<td>Feeding Session Cover Sheet</td>
<td>126</td>
</tr>
<tr>
<td>7.7.2</td>
<td>Motivation to Eat Visual Analogue Scale (VAS)</td>
<td>127</td>
</tr>
<tr>
<td>7.7.3</td>
<td>Physical Comfort VAS</td>
<td>128</td>
</tr>
<tr>
<td>7.7.4</td>
<td>Beverage Pleasantness VAS</td>
<td>129</td>
</tr>
<tr>
<td>7.7.5</td>
<td>Beverage Sweetness VAS</td>
<td>130</td>
</tr>
<tr>
<td>7.7.6</td>
<td>Food Pleasantness VAS</td>
<td>131</td>
</tr>
<tr>
<td>7.7.7</td>
<td>Sick Kids Milk Study Case Report Form</td>
<td>132</td>
</tr>
<tr>
<td>Appendix 8</td>
<td>Pizza Meal Records &amp; Blood Collection Records</td>
<td>135</td>
</tr>
<tr>
<td>7.8.1</td>
<td>Experiment 1: Pizza Meal Records</td>
<td>135</td>
</tr>
<tr>
<td>7.8.2</td>
<td>Experiment 2: Pizza Meal Records</td>
<td>136</td>
</tr>
<tr>
<td>7.8.3</td>
<td>Experiment 2: Blood Collection Records</td>
<td>137</td>
</tr>
<tr>
<td>Appendix 9</td>
<td>Miscellaneous Forms</td>
<td>138</td>
</tr>
<tr>
<td>7.9.1</td>
<td>Experiment 1: Reminder Forms</td>
<td>138</td>
</tr>
<tr>
<td>7.9.2</td>
<td>Experiment 2: Instructions &amp; Map</td>
<td>139</td>
</tr>
</tbody>
</table>
Chapter 1
Introduction

Childhood obesity is one of the foremost public health concerns developed nations presently face. Approximately 1 million and an additional 600,000 Canadian school-aged children are overweight and obese, respectively [1]. Childhood obesity is associated with the development of chronic illnesses in adulthood creating a huge burden on the already stretched health care system. Childhood obesity can lead to the development of type II diabetes (T2D) and metabolic syndrome in their youth that worsens with increasing obesity [2, 3]. The causes of obesity are complicated but the food environment is suggested to be one factor contributing to the increase prevalence in obesity. The consumption of traditional foods such as milk has decreased while consumption of inexpensive, highly processed and palatable foods has increased [4, 5].

Childhood obesity has increased alongside the significant rise in sugar-sweetened beverage (SSB) consumption while milk intake has decreased [4, 5]. Epidemiological studies find strong positive associations between SSB intake and body weight gain and risk of T2D [6] and often attribute this to added caloric intake from added sugars. However, there is little experimental evidence from randomized control trials assessing the relationship between SSBS and T2D because of a lack of resources to carry out a well-powered study [7]. Milk is inexpensive and a good source of high quality nutrients, yet Canadian children and youth (4–16 years old) consume less than Canada’s Food Guide recommended 3–4 servings of dairy per day [5]. Many epidemiological studies demonstrate that frequent dairy intake is associated with lower obesity [8] and T2D [9], but others do not [10, 11]. Short-term studies also show conflicting results and do not always show that milk offers better food intake control than other caloric beverages but more often than not, milk demonstrates that there are benefits in glycemic control [12, 13].

Milk and dairy products may have functional properties as a dietary approach to prevent and manage obesity. Understanding the mechanisms of how milk and dairy products regulate satiety, food intake, and blood glucose has not been explored in children during a critical stage of development. Therefore, the focus of this thesis is on the effect of fluid dairy products on satiety, food intake and glycemic regulation in children.
Chapter 2
Literature Review

2.1 Introduction

To provide a background for this thesis research the following literature review is presented in seven sections. It begins with a brief introduction to the problem of obesity and type II diabetes (T2D) in children and adolescents and is followed by an examination of beverage consumption trends and their relationship to obesity and T2D. The next section explores the components that make up milk and their effects on satiety, food intake (FI) and glycemic control. Finally, an overview of the physiological mechanisms of satiety, FI and glycemic control is followed by a brief presentation of the effect of milk and its components on these parameters.

2.2 Obesity and Type 2 Diabetes in Children and Adolescents

Overweight (OW) and obesity has increased markedly over the last 40 years and now over two-thirds of Canadian adults are OW or obese (OB). Presently in Canada, the prevalence of childhood obesity has tripled over roughly one generation with almost 1 million OW and 600,000 OB school-aged children [1, 14, 15]. One third (32%) of Canadian children and adolescents 5–17 years old were classified as OW (20%) or OB (12%) in 2009 to 2011 [14].

Worldwide trends predict that the prevalence of OW and obesity in preschool aged children in developed and developing countries is expected to increase from 6.7% in 2010 to 9.1% by 2020 [1]. Most striking is that in 2010, 43 million preschool children were estimated to be OW and OB with 92 million estimated to be at risk of becoming OW [1]. The worldwide prevalence of OW and obesity in children and adolescents between 5–17 years old is estimated to be 10%; however, there is a wide discrepancy between regions and show that the prevalence for OW and obesity in the Americas and Europe (20%) is greater than Africa and Asia (10%) [16].

The consequence of childhood obesity is not limited to the association of the development of chronic illnesses in adulthood but can lead to the development of T2D and the metabolic syndrome in their youth that worsens with increasing obesity [2, 17]. Approximately 95% of
Canadian children with T2D are OB at the time of diagnoses with an average age of 13.7 years and 8% are younger than 10 years old [3]. The onset of T2D in this young population sets a course towards a future for a wide range of serious health complications [2]. Unlike type I diabetes identified as an autoimmune disease characterized by the destruction of insulin-producing cells in the pancreas, T2D is the result of insulin resistance where cells fail to respond to the normal actions of insulin resulting in hyperglycemia and subsequently hyperinsulinemia from the continual production of insulin until it can no longer be produced [18]. Primary risk factors contributing to the development of T2D in children include increased weight and lack of physical activity but other factors also include familial history of T2D, hypertension, lipid disorders or diagnosis of acanthosis nigricans or polycystic ovary syndrome [18, 19].

The negative consequence of obesity poses as a major public health concern and is a huge burden to the health care system. The direct and indirect health care cost for Canadian adults with excess weight in 2006 was more than $11 billion [15]. In addition to the medical consequences of obesity, the psychosocial development of a child can be affected resulting in the lack of self-esteem, which is associated with higher rates of sadness, loneliness, and nervousness [2, 20]. Moreover, they are also more likely to partake in high-risk behaviours such as smoking or consuming alcohol, which can exacerbate their health complications [20]. As a result, there is a great need to address the childhood obesity epidemic where there is an opportunity to mediate and reverse the trend of OW/OB children and youth from becoming OB adults. It is recognized that the increased prevalence of obesity is accompanied by changing environmental factors conducive to an increase in energy intake and a reduction in energy expenditure. The increase in “screen time” reduces the level of energy expenditure [21] and the food environment contributes significantly to increases in energy intake.

Many aspects of the food environment have been targeted as being at fault, including readily access to inexpensive food and beverages, eating out of home, large serving sizes, reduced in-home meal preparation and family mealtimes, fat and sugar in processed foods and beverages, and food advertising [22, 23]. Beverages, more specifically the increased consumption of sugar-sweetened beverages (SSBs) have been identified as a primary factor contributing to the trend of
OW and obesity in children [23, 24]. Moreover, parallel to the increase in obesity and related metabolic diseases and its association with the rise in consumption of SSBs including fruit and soft drinks [24–26], is a decrease in milk consumption [27, 28].

2.3 Beverage Consumption by Children and Adolescents

More than 80% of total water intake comes from fluids including water, other non-caloric and caloric beverages [29]. In addition to its energy contribution, beverages may be a source of nutrients. Both Canadian and American children and adolescents receive close to one-fifth of their daily calories from beverages such as milk, fruit juice, fruit drinks and regular soft drinks [5, 30]. According to the 2004 Canadian Community Health Survey, water, milk and fruit juice accounted for about 60% of beverages consumed by children and youth between 9–18 years old, while consumption of SSBs including soft drinks and fruit drinks made with less than 100% fruit juice accounted for 40% [5]. The consumption of SSBs particularly soft drinks also increases with age, and is associated with increased risk for OW and obesity in children [5, 31].

Beverages have been implicated as casual in the obesity epidemic for two reasons. First, SSBs contain high-fructose corn syrup (HFCS) rather than sugar (sucrose) [32]. Second, it has been proposed that beverages have weak satiety properties and result in poor dietary compensation. Although the notion that the substitution of HFCS for sucrose in beverages causes obesity was proposed in 2001 [32], it has not been supported. The HFCS used in beverages is very close to the same fructose and glucose content as sucrose and gives a similar metabolic response and effect on FI [33]. In addition, several meta-analyses did not show differences in the associations between obesity and SSBs between countries using HFCS from those using sucrose in beverages [34]. The key factor associating with OW and obesity is excess energy intake.

2.3.1 Sugar-Sweetened Beverage Consumption, Obesity and Type II Diabetes

Increased consumption of SSBs has risen in parallel to the increase prevalence in obesity and T2D. Sugar-sweetened beverages now constitute a greater amount of total daily added sugar intake than it did in the past two decades [35]. Caloric sweeteners usually added to beverages comprise of sucrose (50% glucose and 50% fructose) or HFCS (most often 45% glucose and
55% fructose), which are used in a range of beverage products including soft drinks, fruit drinks, energy drinks, and vitamin water drinks. The relationship between the increased consumption of SSB and weight gain and risk for T2D has gained a great deal of interest to seek counter measures to mediate these outcomes. Epidemiological studies have found strong positive associations between SSB intake and body weight gain and risk of T2D [6]. Large prospective cohort studies established temporal patterns that further confirmed that consistently high levels (> 1/day) of SSB intake led to greater weight gain at a 4 and 8-year follow-up and greater risk of developing T2D [36]. Furthermore, higher consumption of SSBs during childhood or adolescence was predictive of a trajectory for weight gain into adulthood [37, 38].

Randomized control trials investigating the relationship between SSB and body weight gain have been few, and some show that reduced SSB consumption decreased body weight by reducing total energy intake [39, 40]. These short-term feeding studies ranging from 3-weeks [40] to 10-weeks [39] in duration revealed that consuming HFCS and sucrose, respectively increased body weight compared to a control group consuming beverages with calorie-free sweeteners. However, in a 1-year intervention trial in OW and OB adolescents, those regularly consuming SSB but with a reduced intake showed a smaller increase in BMI at 1-year compared to the higher intake control group but reduced SSB intake did not improve overall body composition over the two-year period [41].

Although there is little experimental evidence from randomized control trials assessing the relationship between SSBs and T2D because of cost and feasibility, well-powered prospective cohort studies show strong and consistent associations that established a dose–response relationship [7]. Furthermore, short-term mechanistic studies using T2D biomarkers such as insulin resistance and chronic inflammation provide a biological rationale and causal relationship [42]. In addition to an increase in body weight and risk for T2D, there is rising evidence that higher SSB consumption leads to development of other chronic diseases, including hypertension, dyslipidemia and coronary heart disease [42].
2.3.2 Milk Consumption, Obesity and Type II Diabetes

In contrast to SSBs, epidemiological studies show higher milk and dairy consumption is associated with lower obesity [8] T2D [9] and metabolic syndrome [9, 43]. Cross-sectional epidemiological studies report that regular consumption of dairy products is associated with healthier body weights and lower risk of T2D in adults [44, 45]. Most of these studies attribute consumption of low-fat dairy to the inverse relationship between milk and dairy intake and the incidence of obesity and T2D [46] because similar trends were not observed with high-fat dairy products. The results from observational studies have mostly been inconsistent and may be due to a variety of factors including study populations [47, 48], ethnicities [49], approaches used to measure intake that often does not include regular intake [50], variation in composition of milk and dairy products [10, 51], and the ease of detecting associations between diet and health markers when items are more frequently consumed than when they are less consumed. Additionally, a healthier diet pattern is not necessarily marked by frequent dairy consumption, but rather is one aspect that makes up an overall healthier diet.

Dietary patterns that include increased consumption of low-fat dairy products, incorporated with increased intakes of whole grains, vegetables and fruits also have an inverse relationship with the risk of weight gain [8], obesity [3, 9] and the incidence of T2D [52, 53]. However, long-term randomized experimental trials completed on children and adults are few and provide conflicting results. Some fail to show consistent benefits with increased consumption of dairy products in ad libitum diets on body weight, adiposity, waist circumference, cardiovascular risk marker [54] or characteristics of metabolic syndrome. Conversely, others find positive outcomes with increased dairy consumption and less adiposity and body weight [55–57]. These differences can also be attributed to the varying study designs, duration of study, sample population and size, selection of dairy, and the frequency of dairy consumption before the study.

The possible relationship between dairy intake and symptoms of the metabolic syndrome has garnered much interest as a strategy to assist in the regulation of energy balance and metabolism. While attention has primarily been focused on the benefits of proteins [58–60] as the major driving force behind the beneficial effects of milk, other components including lactose
[61], calcium [55, 62], medium-chain triglycerides [63], and conjugated linoleic acids [64, 65] may also influence body weight and metabolic control.

Epidemiological studies rely heavily on personal food recalls, while intervention studies typically use treatments or ingredients not part of the usual diet. Under those circumstances, the observed effects of dairy cannot be isolated to determine the potential role it has on physiological mechanisms. However, there is sufficient epidemiological data to encourage the testing of the hypotheses on the physiological actions of dairy, primarily factors impacting FI and metabolic regulations, including time, frequency of consumption, amount and composition.

2.4 Milk

Milk is described as a complex emulsion dispersed in a continuous aqueous phase with dissolved lactose, proteins, vitamins, minerals and other components. When raw milk is allowed to stand, the natural process referred to as creaming occurs, where fat globules rise to the surface [66]. A similar process occurs through centrifugation that is used to separate skim milk and cream called skimming [66]. Milk is homogenized to reduce the size while increasing the number of fat globules to attain creaming stability [66, 67]. Milk undergoes pasteurisation to destroy pathogens and reduce vegetative bacteria but it also inactivates enzymes responsible for causing milk to become rancid [66, 67]. Fluid milk is often combined with other ingredients to create flavoured milk such as chocolate milk, which is amongst the most popular type of flavoured dairy beverage [68]. Milk can also be fermented to develop other products including yogurt, buttermilk, sour cream and kefir.

Milk is comprised of a complex mixture of proteins (whey protein and casein), fats (saturated, mono- and poly-unsaturated fatty acids) and carbohydrates (lactose). It also contains micronutrients that include vitamins, minerals and biologically active substances, such as immunoglobulins, enzymes, cytokines, hormones and growth factors. As a result, the health benefits of milk may be derived from the function of the individual components but also their collective action when consumed from milk.
Milk is traditionally recommended for consumption because of its nutritional properties contributing several essential nutrients. It is a significant source of micronutrients that contribute more than 10% of the requirements for vitamin A and B12, thiamine, calcium, phosphorous, magnesium, zinc and potassium [69]. In addition, dairy proteins and peptides provide essential amino acids that improve the bioavailability of minerals including calcium, magnesium, zinc, selenium and iron [70]. Many Canadians fall below the recommended intake levels for many nutrients present in milk such as calcium, vitamin D, potassium and magnesium [71]. For this reason, the absence of milk intake would lead to difficulties for individuals to conform to the nutritional requirements for these nutrients [72]. However, it is largely the protein content and metabolic function beyond its provisions of amino acids that may provide a causal link between dairy consumption and better body weight management, lower T2D and metabolic syndrome.

2.4.1 Milk Proteins: Overview

Milk proteins have properties that may provide an explanation for the association between higher dairy intake and lower body weights, and decreased risk of developing hyperglycemia and T2D [59]. The reasons for the observed benefits of increased dairy consumption and the lower prevalence of obesity and chronic illness continue to be unclear; however, the physiologic action of milk protein has been suggested to explain these benefits [73, 74]. The main protein factions in milk, whey protein and casein, suppress short-term FI through metabolic regulation by stimulating hormones that regulate FI and glucose utilization [59, 60].

Milk proteins are a leading source of bioactive peptides with a growing number found in milk protein hydrolysates and fermented dairy products. At least 26 bioactive peptides have been identified that are encoded in milk proteins, which are released in part by the fermentation of milk and the maturation of cheese that enhance these dairy products [75].

The protein content of cow’s milk is 80% casein and 20% whey proteins and each component is composed of complex proteins with various characteristics [67, 76]. Bovine casein (CN) consists of four families with an approximate breakdown of 40% αs1-CN, 10% αs2-CN, 45% β-CN and the remaining κ-CN [76]. Casein is mostly found in a colloidal particle recognized as
the casein micelle and its biological function is to transport the highly insoluble liquid form of calcium phosphate as well as to form a clot in the stomach for more efficient nutrition [77]. When proteolytic enzymes break down caseins it produces bioactive peptides that affect cardiovascular, immune, nervous and gastrointestinal (GI) systems [78]. In the process of cheese-making, caseinomacropeptide and glycomacropeptide, the glycosylated form of caseinomacropeptide, both found in κ-CN are hydrolyzed into para-κ-casein by chymosin; thus becoming whey constituents that are removed with the liquid whey.

Whey proteins constitute the lesser fraction of milk proteins that remain soluble in milk serum or whey after precipitation of caseins at pH 4.6, and this group includes α-lactalbumin, β-lactoglobulin, serum albumin, immunoglobulins, lactoferrin and other secretory components [76, 79]. The health benefits of whey proteins are the result of peptides released during digestion. Peptides derived from whey protein have physiological functions that include modulating blood pressure, inflammatory mechanisms, hyperglycemia and FI regulatory systems. Moreover, these actions are not confined to the individual components of whey proteins and peptides; rather, they act synergistically together and with other whey constituents such as calcium. Consuming milk with calcium has been shown to attenuate weight and fat gain and lower blood pressure to a greater extent than calcium supplementation alone [55].

2.4.1.1 Milk Proteins: Physiological Properties

Casein is considered a “slow” protein, while whey protein is considered a “fast” protein. These categorizations are based on their rate of digestion and absorption demonstrated by plasma amino acid concentrations and the addition to protein synthesis [80]. When humans ingest whey proteins, plasma amino acids rapidly rise reaching peak levels 40 min to 2 h after consumption then returning to baseline concentrations after 3 to 4 h. Conversely, casein produces a lower plasma amino acid concentration that rises slowly and has a prolonged plateau that lasts up to 7 h after ingestion [80]. The rapid absorption of whey protein is the result of whey protein being a soluble protein. In contrast, the slow absorption of casein is because it clots in the stomach, which delays gastric emptying, therefore producing a slower release of amino acids [80, 81].
The physical differences between casein and whey proteins contribute to their functional properties in food systems and physiological effects when consumed.

Numerous studies reveal that milk proteins increase satiety but it is not always predictive of subsequent FI because of factors such as protein source, quantity and time of measurement [82]. A randomized, single-blind study in healthy young adults revealed that a breakfast containing 25% energy from casein was more satiating up to 3 to 4 h later than a breakfast containing 10% energy from casein, but there were no differences in FI at lunch [83]. Similarly, while there were no differences in FI, subjective appetite scores were reduced after the consumption of whey protein (15 g) that was 10% of the energy content in a breakfast for up to 4 h compared to casein and soy [84]. Conversely, when treatment and meal intervals were reduced, a number of studies showed that milk proteins increased satiety as well as decreased FI [60, 82].

Experimental data show that consuming preloads of whey protein (45–50 g), which is beyond the usual amount found in a serving of milk (250 mL, 9 g protein) reduced FI more than casein at a meal consumed 30–90 min later [82], but casein reduced FI more than whey protein at 180 min [59]. Although individual milk proteins showed benefits in FI regulation when provided at doses that exceed the amount found in a serving of milk, it provides little evidence that milk consumed in its usual amounts contribute to FI regulation. However, milk consumed in larger quantities (360–500 mL, 18 g protein) has functional implication for FI control through the combined effect of whey in early, and casein in later satiety. For instance, consuming milk (600 mL, 21 g protein) with a toast and jam breakfast reduced FI and hunger ratings, and increased fullness over 4 h to lunch time more than a fruit beverage (600 mL, 0 g protein) [85].

Few considerations have been given to the potential role of milk consumption in controlling glycemia. However, many studies have shown the effect of whey protein consumed alone, as a beverage or consumed with carbohydrate improves postprandial glycemic control [60, 86–88], primarily by its effect on insulin. For example, individuals with T2D that consumed a carbohydrate meal with the addition of whey protein (18 g) had 21% lower postprandial blood glucose response and 57% higher plasma insulin concentration over 120 min [86]. Furthermore, preloads of carbohydrate (25 g) given with whey protein (18.2 g) produced a 50% greater
increase in insulin response compared to milk or cheese in healthy subjects, suggesting that
whey protein is the leading insulinotropic factor [87]. The greater insulinotropic effect of whey
protein may be the result of its rapid digestion [58, 73, 74], because the casein and fat content of
milk and cheese produce a much smaller effect on insulin but both reduce postprandial glycemia
[88]. More recent studies show that reduces in postprandial glucose is reduced by not only
insulin but also by insulin independent mechanisms of milk proteins [60, 88, 89].

2.4.2 Milk Fat: Overview

Milk fat is generally recognized as a risk factor for cardiovascular diseases; thus, leading to the
development of reduced-fat and fat-free milk and dairy products. The effect of milk fat remains
somewhat contentious, as there are opposing views on its overall health effects [90] and
investigations in dairy consumption related to higher serum lipid levels and cardiovascular
disease mortality have limitations [91, 92].

Milk fat, for many years, has been associated with increased blood cholesterol concentrations,
but studies have found a reduction in cholesterol and other blood lipids for diets containing milk
and fermented dairy products [93, 94], suggesting that these products may have components that
counteract the effect of saturated fatty acids (FA) [93, 95]. Additionally, milk FA have
important metabolic functions and may have a role in short-term effects on hormonal responses
that regulate satiety, FI and glycemia. Milk fat is increasingly being recognized as being neutral
[96] or even beneficial in the control of blood lipids and was negatively associated with
cardiovascular risk factors [97].

Raw cow’s milk typically contains 4% total fat but the concentrations of FA can vary
considerably depending on the breed of cow, season, type of feed, nutritional status, stage of
lactation, age and overall health of the cow, and the time during milking when the sample is
taken [67, 96]. Ordinarily, the total fat content of milk is 33 g/L and the lipid content consists of
roughly 95% triglycerides, 1–2% diglycerides, < 1% monoglycerides, < 0.5% cholesterol, ~ 1%
phospholipids and free FA [67, 96]. Fatty acids vary in length and are classified accordingly:
short-chained (C4:0), medium chained (C6:0-C10:0) and long-chained (> C12:0) [93].
Furthermore, milk fat is made up of approximately 65% saturated, 30% monounsaturated and 3% polyunsaturated FA [73]. The differences in chain length and degree of unsaturation of the FA may account for the varying effects of milk fats on satiety, FI [99] and glycemia [89, 100].

### 2.4.2.1 Milk Fat: Physiological Properties

Milk fat regulates the activation of the ileal brake, which is an inhibitory distal to proximal feedback mechanism controlling the transport of food through the digestive tract improving the digestion and absorption of nutrients [101]. As a result, gastric emptying is delayed, small intestinal transit time is increased, gastric and pancreatic secretions are reduced along with hunger and FI [102]. Fat infused in the duodenum triggers the release of GI hormones peptide tyrosine tyrosine (PYY) and cholecystokinin (CKK) involved in satiety and FI control, but the effects are not limited to one region of the small intestine and increases of satiety and reductions in FI are observed to be greater when fat is infused into the ileum [88, 101]. These effects may be the result of the physiochemical properties of FA that include chain length and the degree of saturation. Fatty acids with chain lengths greater than C10 demonstrated greater inhibition of gastric emptying that would contribute to greater satiety [102]. Additionally, a double-blind, randomized, crossover study in healthy subjects revealed that when canola oil (C18:2) and safflower oil (C18:2) were infused into the ileum, increased fullness and reduced hunger compared to shea oil (C18:0) [101]. These results suggest that where these FA naturally occur in dairy, it may add to ileal brake phenomenon that fat-free dairy products do not. Furthermore, intervention studies have yet to explore full-fat against fat-free milk on satiety, FI and glycemia; however, palmitate, the leading FA in milk has shown to increase hepatic glucose production at low to normal blood glucose concentrations [103].

### 2.4.3 Milk Carbohydrate: Overview

Lactose is the main source of milk carbohydrate that is dissolved in the aqueous phase of whole milk with whey proteins and other minerals [67]. Its concentration in whole milk is approximately 53 g/L [96]. There are also minute amounts of carbohydrates in fresh milk including glucose, galactose and oligosaccharide.
Lactose is a disaccharide sugar composed of glucose and galactose. Regular pasteurization of fluid milk has little effect on lactose but certain processes used to extend shelf life such as ultra-high temperature pasteurization or spray-drying can alter its structural arrangement. Referred to as isomerization, reduces the lactose content and converts lactose to lactulose, which prevents digestive discomfort in individuals with lactose intolerance [67, 96].

2.4.3.1 Milk Carbohydrate: Physiological Properties

Lactose is hydrolyzed by the enzyme lactase to glucose and galactose, which are absorbed in the small intestine. Individuals deficient in lactase experience lactose intolerance and very often avoid dairy products. However, it has been demonstrated that one cup of milk can be consumed without experiencing symptoms. Moreover, including milk as part of a meal or choosing fermented dairy alternatives products can alleviate the symptoms [67, 96, 104].

Lactose contributes to the low glycemic effect of milk because of the lower glycemic response it produces compared to sucrose and glucose. This may be because lactose is not readily absorbed compared to sucrose or that galactose brings about a lower glycemic response than fructose [105]. Experimental studies show that healthy adults consuming 50 g of galactose did not produce a rise in blood glucose [106]. Furthermore, OB adults consuming 56 g of lactose had lower glycemic and insulin responses and lower FI at 180 min than the same amount of glucose [61]. A study that compared the glycemic indices of 25 g carbohydrate of white bread, lactose and whole milk revealed that the glycemic index of whole milk was significantly lower than both white bread and lactose but the insulin index was significantly greater for whole milk compared to lactose [107], suggesting that there were other factors within whole milk that led to the stimulation of insulin.

2.4.4 Milk and Milk Products

Milk is used to make a variety of products that include flavoured milk and yogurt to name a few. Flavoured milk, in particular, chocolate milk is the predominant type of flavoured milk consumed amongst Canadians [68]. Chocolate milk typically contains 1–3% cocoa powder, 3–6% sucrose and other stabilizing agents. Yogurt on the other hand is fermented by a blend of
*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* [67]. Various ingredients can be added and processes used to make a variety of yogurt products including sweetened stirred style yogurt, fruit-on-the-bottom set style, frozen yogurt, probiotic yogurt and yogurt beverages [66]. These products have similar but slightly altered nutrient profiles compared to milk and may have varying effects on FI, satiety and glycemic control.

2.4.4.1 Flavoured Milk

Milk, as a result of added sugar used in flavoured milk, has become of concern as a contributor to weight gain, obesity and T2D in children. A prospective study comparing flavoured milk consumers with non-consumers found that OW/OB children who consumed flavoured milk had a larger increase in body fat than non-consumers over a two-year period, but this relationship was not observed in NW children [108]. In contrast, in an alternate study, plain and flavoured milks were positively associated with nutrient intake and not associated with negative weight outcomes for children and adolescents [109].

There are, however, few experimental studies comparing the short-term effect of milk and flavoured milk consumption on FI and glycemic control. One study investigating the short-term effects of commonly consumed beverages on FI and glycemic response in healthy young adults found that pre-meal consumption of 500 mL of chocolate milk (340 kcal) reduced FI 30 min later compared to a water control (0 kcal) but similar reductions were not observed after consuming 2% milk (260 kcal), suggesting that the caloric content was the important factor [12]. However, 2% milk resulted in smaller increases of pre-meal and total blood glucose than chocolate milk but post-meal blood glucose was lower after both beverages compared to water suggesting that the milk protein content in the beverages drove this reduction [12].

2.4.4.2 Yogurt

Traditional yogurt has a similar nutrient profile to milk but many current brands have a higher concentration of milk proteins, which can contribute to lower FI, increased satiety and better glycemic control. Regular consumption of yogurt associates with better diet quality and metabolic profiles in adults [110]. Yogurt in both beverage and semi-solid form lowered hunger
and increased ratings of fullness compared to a fruit drink and dairy fruit drink but subsequent FI at a meal 120 min later was not different between preloads in healthy young adults [111]. In addition, yogurt, like milk, when consumed as a snack attenuates postprandial glucose excursions better than a fruit beverage [112].

2.5 Regulation of Food Intake and Glycemia

Short- and long-term feeding behaviours and glycemic control are regulated by complex interactions among neural, metabolic, peripheral and hormonal signals entering the brain. The ingestion and digestion of dairy stimulate signals regulating satiety, FI and metabolisms in a variety of ways as a result of the unique composition of dairy. A short review of the mechanisms controlling satiety, FI and blood glucose is presented as background then followed by the examination of the effects of milk on these mechanisms.

2.5.1 Physiology of Food Intake Regulation

The regulation of short- and long-term FI involves a complex physiological and neurological process. The central nervous system (CNS) regulates FI by integrating signals arising from sensory properties of food, mechanical and chemical receptors in the gut, circulating metabolites and hormones. The hormones that originate from the GI tract, liver and pancreas are integrated by the hypothalamus in the brain, which translates the signals into information used for regulating when, what and how much food is consumed [113]. These physiological mechanisms are modulated by pre- and post-absorptive signals in response to food consumption. Pre-absorptive signals are the result of gastric distention, gastric emptying and the secretion of GI hormones responding to ingested food. Post-absorptive satiety signals arise as nutrients are digested and enter circulation.

2.5.2 Hormonal Regulation of Satiety and Food Intake

The GI tract is the first organ affected by nutrient ingestion. It releases more than 20 regulatory peptides during nutrient ingestion through a complex neural network that exert anorexigenic (appetite suppressing) and orexigenic (appetite stimulating) effects in the hypothalamus. While many of these peptides are synthesized in the brain, this section focuses on the GI tract as a
major source of these hormones in the peripheral circulation. When food is consumed, hormones synthesized and secreted from the stomach, intestine and pancreas trigger signals that decrease FI. Macronutrients stimulate the release of pancreatic hormone, insulin and intestinal peptides including glucagon-like peptide-1 (GLP-1), PYY, CCK and ghrelin.

2.5.2.1 Pancreatic Hormones

Insulin is the principal metabolic and endocrine hormone secreted by the β-cells from the pancreas in response to macronutrient intake. It is a vital satiety signal that modulates FI more than other GI satiety hormones [33, 114, 115]. Plasma glucose is the strongest predictor for insulin secretion, which affects short-term FI and satiety both directly in the hypothalamus and indirectly by its effects on blood glucose [116–118]. Insulin secretion is enhanced by increased glucose metabolism in pancreatic β-cells leading to the products of glycolysis that activate membrane depolarization in the β-cells to release insulin [119]. Insulin sensitive glucose transporters, GLUT-4 and GLUT-8, detected in appetite-regulating areas of the brain including the hypothalamus are translocated to the plasma membrane of glucose-sensing hypothalamic neurons, and increase their sensitivity to blood glucose concentrations, therefore increasing cellular uptake of glucose and suppressing appetite [120]. Insulin in the brain also plays a long-term role in the regulation of FI and body weight. Animal studies showed that insulin delivered to the hypothalamic region of the brain inhibited FI and produced permanent body weight loss (leptogenic action) [113, 121, 122]. But when insulin antibodies were injected into the ventromedial hypothalamus of rats, FI increased resulting in body weight gain [123] demonstrating the significant role insulin plays in energy homeostasis.

2.5.2.2 Gastric Hormones

Produced in the stomach by the A-cells, ghrelin is the single orexigenic hormone. Its effects are exerted via the arcuate nucleus of the CNS to increase gastric motility and emptying, and enhance appetite and FI [124]. The arcuate nucleus of the hypothalamus is not protected by the blood-brain-barrier and is able to respond to peripheral ghrelin concentrations; therefore a rise in plasma ghrelin stimulates appetite and FI by binding to its receptors (GRLN-R) in the arcuate
nucleus [125]. Ghrelin is associated with short-term mealtime hunger and meal initiation as a result of circulating ghrelin concentrations rising before a meal and decreasing proportionally to the caloric content of a meal [126]. Ghrelin also increases FI by raising the number of meals initiated without changing their size and stimulates several appetitive feeding behaviours [127].

Two main forms of ghrelin found in plasma are acyl and des-acyl ghrelin [128]. Plasma concentrations of acyl ghrelin together with total ghrelin (acyl and des-acyl) decline significantly after ingestion of nutrients; an indication that the short-term regulation of acyl ghrelin responds similarly to total ghrelin [128]. After a meal, plasma ghrelin concentrations decrease as a result of appetite suppressing hormones [125].

2.5.2.3 Gastrointestinal Hormones

The GI tract is the first tissue affected by nutrient intake before interacting with the pancreas, liver, muscle, adipose tissue and CNS in regulating FI. The GI hormones regulate satiety and FI through their effect on insulin secretion and gastric emptying [129, 130]. Gastric emptying is inhibited through the actions of GLP-1, CCK and PYY when they exert their satiety and metabolic effects. CCK is released by endocrine I-cells in the small intestine, specifically the duodenum and jejunum [131]. In the distal parts of the GI tract, GLP-1 [132] and PYY [133] are secreted from L-cells, which cross the blood-brain-barrier and directly transmit signals inhibiting appetite and gastric emptying.

Macronutrients activate the release of each hormone in varying capacities. Dietary fat and protein primarily stimulate the release of CCK but not carbohydrate [134–136]. While all three macronutrients, protein, fat and carbohydrate stimulate the release of PYY and GLP-1. However, the degree in which PYY is released depends on the macronutrient, size of the meal and the time-course [88,137]. Additionally, several studies point to increased GLP-1 responses after protein compared to other macronutrients [88, 138].

2.5.3 Regulation of Glycemia

Glycemic control depends on controlling postprandial glucose. High postprandial glucose has been independently related to adverse metabolic outcomes including increased oxidative stress
and endothelial inflammation, abnormal vascular reactivity, glycation, and hypercoagulability [139].

Glycemic control is regulated by complex interactions between neural, metabolic and hormonal signals. The sources plasma glucose concentrations are derived from include endogenous glucose from gluconeogenesis and glycogenolysis, and exogenous glucose from food consumption [140]. At fasting, glycogen is converted to glucose in the liver through the process of glycogenolysis, while gluconeogenesis utilizes lactate and amino acids to produce glucose [140]. After food consumption, postprandial glucose concentrations are affected through carbohydrate absorption, insulin secretion, glucagon and other GI hormones, the combined effects of glucose metabolism and the rate of gastric emptying [140, 141]. Additionally, timing, quantity and composition of the meal heavily influences the magnitude and time glucose concentration reaches peak levels [141].

2.5.4 Hormonal Regulation of Glycemia

2.5.4.1 Pancreatic Hormones

Insulin plays a key role in regulating glycemic control. It is secreted in response to increased blood glucose as a result of food consumption. The primary role of insulin is to stimulate glucose uptake by binding to its receptors in adipose, liver and muscle cells. The constant rate of glucose uptake in the peripheral tissues and endogenous glucose production in the liver prevents blood glucose elevation [142]. Insulin assists in the regulation of postprandial glucose through three methods. First, insulin stimulates GLUT4 receptors in the insulin-sensitive peripheral tissues, primarily the skeletal muscle to increase glucose uptake [143]. Next, it acts on the liver to stimulate glycogenesis. Lastly, signals from insulin are sent through the portal vein to the liver to inhibit the production and release of glucose through glycogenolysis and gluconeogenesis [144].

Glycogenolysis and gluconeogenesis are regulated by the hormone glucagon, which is the major counterpart to insulin. Glucagon is secreted in the pancreas by the α-cells and maintains basal plasma glucose concentrations within a normal range during fasting and is suppressed after food...
consumption. The dominant stimulator of insulin is carbohydrate while protein is found to stimulate the release of both insulin and glucagon [145, 146]. Protein may have an important role in long-term glycemic control by reducing and preventing hypoglycemia as a result of protein-induced glucagon secretion to prevent insulin-induced hypoglycemia. Fat intake on the other hand does not independently stimulate insulin secretion, but when it is consumed in combination with a carbohydrate, it may significantly affect plasma glucose and/or insulin responses in healthy humans [147].

2.5.4.2 Gastric Hormones

Ghrelin has a significant role in regulating glucose homeostasis [148]. It has an inverse secretory pattern in relation to insulin [149, 150] and glucose [151] indicating the inhibitory feedback between ghrelin and insulin [148]. Insulin has also demonstrated its ability to suppress circulating ghrelin concentrations, independent from changes in glucose concentrations [151]. Macronutrient content of a meal can have significant effects on postprandial ghrelin response. In particular, carbohydrates have the strongest effect on decreasing postprandial ghrelin concentrations [152]. Additionally, milk-based proteins consistently show reductions in ghrelin levels [153]. However, high-fat meals have varying effects and have demonstrated to increase [152] as well as decrease [154] ghrelin concentrations. When ghrelin concentrations are decreased as a result of high fat intake, it has been shown to have a slower return to baseline than after a high carbohydrate meal [155].

2.5.4.3 Gastrointestinal Hormones

Gastrointestinal hormones such as GLP-1 contribute to glycemic control by stimulating insulin secretion and glucagon suppression to inhibit hepatic glucose production and lowering blood glucose concentrations [129, 130]. GLP-1 is a strong insulin stimulator and does so in a glucose-dependent manner, but the presence of dipeptidyl peptidase IV (DPP-IV) enzyme rapidly cleaves these incretins and deactivates the hormones, leaving a small proportion of the secreted GLP-1 to reach the pancreatic β-cells to stimulate insulin secretion [156]. Besides maintaining blood glucose by affecting insulin secretion [157, 158], GLP-1 receptors (GLP-1R) are activated in the periphery to enhance hepatic insulin action to replenish hepatic glycogen stores [159] and
to increase removal of meal-derived glucose by activating neurons in the hypothalamus [160]. Glucose disposal is also enhanced by PYY in the portal vein when hepatic and pancreatic vagal afferents are stimulated, thereby, heightening portal-mediated glucose clearance [161, 162].

2.6 Milk in the Regulation of Satiety, Food Intake and Glycemia

Frequent dairy intake has previously been reported to be associated with lower risk of obesity [8] and T2D [9]. However, experimental studies using body weight change or FI as outcomes have yet to show convincing evidence of a diet rich in milk on body weight and adiposity [54, 55, 111]. Diets containing whey protein, a component of milk [53], may suppress hunger and FI, thus reducing fat deposition and improve insulin sensitivity; however, this is not necessarily a confirmation that milk will have the same effect. Individual components of milk have been explored on their effect on short-term appetite and FI but limited investigation have been done in milk as a whole entity on satiety, FI and glycemic control.

2.6.1 Satiety and Food Intake

Short-term studies are most suited for measuring satiety and FI and often utilize a preload design. In addition to timing of the meal, calories are usually primary factors determining short-term FI [12], but there is evidence that composition of calories can also be a factor when compared to caloric sweeteners [85, 163, 164]. Calorie content and time to the next meal are clear determinants of satiety. In a study comparing isovolumetric (500 mL) amounts of commonly consumed beverages in healthy young adults, all caloric beverages increased satiety during the pre-meal period [12]. However, only chocolate milk (340 kcal) and infant formula (368 kcal), the beverages with the greatest calories, reduced ad libitum FI at 30 min compared to water but were similar to milk (260 kcal) and soy beverage (200 kcal). Ad libitum FI was similar between all beverages at 120 min indicating that mealtime interval also determines the effect of calorie content on later short-term FI [12].

The importance of beverage composition has been shown more clearly when isocaloric beverages are compared. A study in healthy young men (n = 22) comparing isovolumetric (500 mL) and isocaloric (215 kcal) amounts of chocolate milk (13 g protein, 36 g CHO) and regular
cola (0 protein, 53 g CHO) revealed that chocolate milk decreased subjective ratings of hunger and prospective consumption, and increased satiety and fullness more than cola [163]. Furthermore, ad libitum FI measured at 30 min was 4% less after chocolate milk than cola but the difference was not significant. The protein content of chocolate milk may explain the increased satiety after chocolate milk, but the minimal effect on FI may be the result of the low energy content of the beverages and timing of the meal [163]. Another study compared isovolumetric (600 mL) and isocaloric (254 kcal) beverages of skim milk (25 g protein, 36 g lactose, <1 g fat) to a fruit drink (<1 g protein, 63 g sugar, <1 g fat) on subjective appetite and FI was measured 240 min later with an ad libitum lunch [85]. Overweight adults (n = 34) consumed each beverage with a fixed energy breakfast (460 kcal), and subjective appetite scores revealed that satiety ratings before the ad libitum meal were higher and FI was lower by 8.5% after skim milk than fruit drink [85]. A recent crossover study in obese adults (n = 24), compared isovolumetric (500 mL) amounts of semi-skimmed milk (950 kJ), regular cola (900 kJ), diet cola (7.5 kJ) and water on subjective appetite, ghrelin, GLP-1, GIP and ad libitum energy intake 4 h after consuming the test beverages [164]. Although differences in energy intake were not apparent among beverages, milk increased satiety, GLP-1 and GIP responses compared with regular cola consistent with the effect of protein ingestion. Ghrelin reduced by 20% after both milk and regular cola compared to water, consistent with its role as primarily an energy sensor [164].

Thus, the hypothesis that the effect of milk on satiety and FI is primarily mediated through the effect of milk proteins on the release of satiety hormones is supported, but there is an inadequate amount of studies investigating how consumption of milk in its whole influences short-term FI and appetite not only in adults, but especially in children.

2.6.2 Glycemic Control

The inverse relationship between increased milk consumption and T2D is well documented in epidemiological studies [9, 44, 45], but experimental studies investigating the effect of milk consumed alone or in meals on glycemic control before and after meals have been sparse.
Short-term experimental studies in healthy young adults show that pre-meal consumption of milk 30 and 120 min prior to an ad libitum meal reduce postprandial blood glucose concentrations compared to a water control, but is similar to other caloric beverages [12]. In addition, another study measured blood glucose over 120 min after ad libitum intakes of commonly consumed beverages with an ad libitum meal and showed reduced blood glucose AUC after milk compared to water, orange juice and regular cola but was similar to diet cola [13]. These studies demonstrate that milk consumption before or as part of a meal offers benefits to glycemic control as a result of milk proteins but it is not limited to its ability to stimulate insulin. The interactions among the macronutrient components of milk regulate postprandial glycemia through both insulin and insulin-dependent actions [89]. Therefore, it remains to be determined whether milk consumed prior to and with a meal improves postprandial glycemia in children.

2.7 Summary and Research Rationale

The increasing prevalence of obesity and T2D in children and adolescents is consistent with an increase in SSB consumption and a decrease in milk consumption. Many epidemiological studies point to a strong association between increased dairy intake and lower risk of obesity and T2D but cannot show cause and effect. However, these associations are supported by experimental studies exploring the effects of milk, milk products and their components on physiological mechanism regulating FI and postprandial glycemia. Several experimental studies report that the components of milk result in increased satiety and better post-meal glycemic control, but few studies have examined milk in comparison with other beverages. No studies have reported a comparison of milk with other beverages on short-term FI, satiety and blood glucose control in NW children or OW/OB children. Therefore the research reported herein was designed to fill this gap.
Chapter 3
Hypothesis and Objectives

3.1 Hypothesis

Compared with a sugar-sweetened beverage, fluid dairy products consumed before and with a meal decrease short-term appetite and food intake and improve glycemic control in children.

3.2 Objectives

- To examine the effects of isocaloric (130 kcal) beverages of 2% milk, 1% chocolate milk, 1.5% yogurt drink, a sugar-sweetened beverage (fruit punch) and water control consumed before and within a pizza meal at 60 min on subjective appetite and mealtime food intake in normal weight and overweight/obese 9-14 y old children. (Experiment 1)

- To compare the effects of isocaloric (130 kcal) beverages of 2% milk and fruit punch consumed before and with a pizza meal at 60 min on glycemic response and appetite hormone regulation in normal weight and overweight/obese 9-14 y old children. (Experiment 2)
4.1 ABSTRACT

**Background:** The benefits of milk consumption, compared with other beverages, on short-term food intake (FI) and glycemic control are well documented in adults; however, similar studies have not been reported in children.

**Objective:** To compare the effects of dairy and non-dairy beverages on satiety and FI (Experiments 1 and 2) and on glycemic and appetite hormone responses (Experiment 2) in normal weight (NW) and overweight/obese (OW/OB) children.

**Design:** Two experiments were conducted. In a randomized repeated measures design, Experiment 1 compared the effects of water (control) and isocaloric (130 kcal) amounts of 2% milk, 1% chocolate milk, 1.5% yogurt drink and fruit punch on subjective appetite and FI. Experiment 2 examined the effects of isocaloric (130 kcal) amounts of 2% milk and fruit punch on subjective appetite, FI, glycemic and appetite hormone responses. In both experiments, beverages were given pre-meal and again 60 min later with an ad libitum pizza meal.

**Results:** In Experiment 1 (n = 32), but not Experiment 2 (n = 20), FI was affected by treatments (P < 0.0005) but only chocolate milk and yogurt lowered FI than water. Pre-meal subjective appetite increased and post-meal decreased the most after milk in both experiments. In Experiment 2, milk led to lower pre-meal glucose and higher pre-meal GLP-1 (P < 0.05) than fruit punch in all children. When intake was adjusted to kcal/kg body weight, post-meal glucose (P < 0.04), insulin (P = 0.006) and GLP-1 (P = 0.01) increased, and ghrelin decreased (P < 0.02) more in OW/OB than NW children. Post-meal PYY was higher after milk in all children (P < 0.04).

**Conclusion:** Milk consumed before and with a meal does not reduce FI and subjective appetite more than other caloric beverages, but results in lower pre-meal glucose and more favourable responses in satiety hormones in children.
4.2 INTRODUCTION

The prevalence of obesity amongst children and youth has more than doubled within the last 25 years [15, 165]. While the causes of obesity are complex and multi-factorial, one causative factor for this rise includes the changing food supply, which contributes to reduced consumption of traditional foods and increased intake of inexpensive and highly processed foods and beverages. Parallel to the increase in childhood obesity is the significant rise in sugar-sweetened beverage (SSB) and decrease in milk consumption [4, 5, 166].

Milk is a good source of high quality nutrients, such as calcium and protein, yet Canadian children and youth (9–18 y old) consume less than the Canada’s Food Guide recommended 3–4 servings per day [5]. Observational studies in children and adolescents show associations between increased dairy and calcium supplementation and lower body fat independent of total energy intake [166–168]. A randomized trial comparing high and low milk consumption has provided partial support for these observations. Supplementation of 8–10 y old overweight (OW) children for 16-wk resulted in no changes in fat loss or body weight but glucose tolerance tests revealed that high milk consumption (4 servings of milk/d) reduced insulin AUC compared to low milk consumption (1 serving of milk/d) [169], suggesting that milk may improve insulin action in OW children.

In adults, the benefits of dairy consumption on energy intake and body weight are well documented [9, 12, 13, 42, 85, 170]. Epidemiological studies show an inverse association between higher dairy consumption and reduced body weight [170, 171], incidence of obesity [47, 170] and components of the metabolic syndrome [169]. Short-term experimental trials showed that milk consumption reduces appetite and food intake (FI) compared to a fruit drink [85] and when consumed prior to [12] or within [13] meals reduced post-meal glycemic response compared with other commonly consumed beverages. These effects of milk have been attributed to its components, including milk proteins (whey protein and casein) [60], carbohydrate (lactose) and fat [89].
To date, no experimental studies have reported the short-term effects of fluid dairy products on subjective appetite, FI, and glycemic and appetite hormone responses in children. We hypothesized that fluid dairy products consumed before and with a meal decrease FI, increase satiety and improve postprandial hyperglycemia and appetite hormone responses in children when compared with a SSB. The objective of this study was to examine the effects of isocaloric (130 kcal) amounts of 2% milk, 1% chocolate milk, 1.5% yogurt drink, fruit punch (SSB) and water (control) consumed before and within a pizza meal 60 min later on satiety and FI in normal weight (NW) and OW/obese (OB) children (Experiment 1) and to compare the effects of 2% milk and fruit punch consumed before and within a pizza meal 60 min later on FI, blood glucose, and appetite hormone responses in NW and OW/OB children (Experiment 2).

4.3 PARTICIPANTS AND METHODS

4.3.1 Study Design

Two experiments were conducted using a randomized, controlled, within-subject and repeated measures design. Children received beverage treatments one week apart in a randomized order using a randomized block design, which was generated with a random generator script in SAS version 9.2. In Experiment 1 (n = 32: 16 NW and 16 OW/OB), treatments were 2% milk, 1% chocolate milk, 1.5% yogurt drink, fruit punch and water (control), and in Experiment 2 (n = 20: 10 NW and 10 OW/OB) were 2% milk and fruit punch. One serving (130 kcal) of the beverages was provided 2-h after breakfast (0 min) and a second serving 60 min later with an ad libitum pizza meal. Satiety, blood glucose and appetite hormones were measured at baseline and intervals before and after the meal, and mealtime pizza intake was measured.

4.3.2 Participants

Children between the ages of 9–14 y and with a BMI percentile ranging from the 5th and 85th for NW, 85th and 97th for OW, and greater than 97th for OB age- and sex-specific BMI percentiles [172] were recruited through newspaper advertisements, the Research4kids database at the Hospital for Sick Children and by word of mouth. Children were excluded from the study if they were taking medications, regularly skipped breakfast, were lactose intolerant, had other
food sensitivities or allergies and were unable to follow instructions. Eligible children were invited with their parents for a screening at the Department of Nutritional Sciences, University of Toronto, and informed written consent was obtained from parents and written assent from the child. Height (m) and body weight (kg) were measured to determine the age- and sex-specific BMI percentile using the WHO BMI-for-age growth reference chart [172]. Bioelectrical impedance analysis was used to estimate body fat mass and fat free mass (RJL Systems BIA, 101Q) based on the Horlick equation [173]. Restrained eating was assessed in all children using the Dutch Eating Behaviour Questionnaire [174]. The procedures of the study were approved by the Human Subject Review Committee, Ethics Review Office at the University of Toronto and the Research Ethics Board at the Hospital for Sick Children.

4.3.3 Beverages

Beverages (Table 1) included 1) milk (2% M.F.; Beatrice®, Parmalat, Toronto, ON) because it is the most commonly consumed type of milk amongst Canadians [68]; 2) chocolate milk (1% M.F.; Beatrice®, Parmalat, Toronto, ON) because it is the most commonly consumed flavoured milk [68]; 3) strawberry flavoured yogurt drink (1.5% M.F.; Yoplait®, Aliments Ultima Inc., Longueil, QB) because it is the second most consumed fluid dairy product [68]; 4) fruit punch (Tropical Rhythms®, Grace Foods, Richmond Hill, ON), the SSB and 5) water (control). All beverages except water were isocaloric (130 kcal) and served chilled in an opaque cup. To match for volume of milk (250 mL) and to minimize aftertaste of the beverages, water was provided following chocolate milk (191 mL + 59 mL of water), yogurt drink (173 mL + 77 mL of water) and fruit punch (232 mL + 18 mL of water). The rationale for providing the beverage twice was that the 1-h interval between the beverage and the meal reflects a pattern where children consume a beverage, such as after school while waiting for the main meal. In addition, we have previously reported that consumption of a beverage with interval of 60 min or less between preload intake and a meal has a significant effect on FI at the meal [175].

4.3.4 Experimental Procedures

Procedures are similar to those reported previously [12, 13, 88, 89, 175, 176]. In Experiment 1, sessions took place on 5 separate mornings and in Experiment 2 sessions took place on two
separate mornings. In each session, 1-wk apart, children were given a standardized breakfast so that they would arrive neither hungry nor full. The breakfast (300 kcal) consisted of Beatrice® fat-free skim milk (250 mL, 90 kcal), Honey Nut Cheerios® (26 g, 100 kcal; donated by General Mills, Inc.) and Tropicana Orange Juice® (236 mL, 110 kcal). Participants consumed the standardized breakfast at home following a 12-h overnight fast at their preferred time in the morning (07:00–09:00), and were asked not to consume anything except for water which was permitted for up to 1-h before the session. Sessions began 2-h following the breakfast (09:00–11:00) at the Department of Nutritional Sciences, University of Toronto (Experiment 1) and at the Physiological Research Unit, The Hospital for Sick Children (Experiment 2).

Upon arrival, trained research assistants interviewed children to ensure their compliance to instructions before proceeding. Children completed ‘Motivation to Eat’ and ‘Physical Comfort’ Visual Analogue Scale (VAS) questionnaires, which have been used in previous studies [175, 176] at baseline and during regular intervals (15–30 min) over 145 min. Children were instructed to read each question carefully before marking an “X” anywhere along the 100 mm line anchored by opposing statements at opposite ends of the line that best reflected their feelings at that time. The motivation to eat questionnaire assessed desire to eat, hunger, fullness, and prospective food consumption and was used to calculate average subjective appetite scores [177]. Average appetite score was calculated at each time of measurement for each beverage using the following formula: Average Appetite score (mm) = [desire-to-eat + hunger + (100 – fullness) + prospective consumption] / 4, which reflects the 4 questions of the Motivation to Eat VAS [178].

Children received the first serving of the beverage at baseline (0 min) and were allowed 5 min to drink. The children engaged in a quiet activity such as reading or card and board games [175, 176] until they were escorted to the feeding room, seated in individual cubicles at 60 min and given the second serving of the beverage with an ad libitum pizza meal. Children were asked to remain seated for the duration of the meal, and were instructed to “eat until you are comfortably full” and “drink all of the drink” over the duration of 25 min. Children consumed the entire beverage and ate the pizza in any order they preferred. Pizzas were baked at 220°C for 8 min
and served fresh every 8 min. Two varieties, pepperoni and three-cheese, of Deep ‘N Delicious 5” diameter pizzas (McCain Foods Ltd, Florenceville, NB, Canada) were served. Each tray contained 3 pizzas (~540 kcal); two of their first choice and one of their second choice. Cooked pizzas were cut into four equal pieces, arranged in a non-uniform fashion and weighed (g) before serving; the amount left after the meal was subtracted from the initial weight. Each variety of pizza was weighed separately, and energy consumed (kcal) at the meal was calculated using manufacturer information. Sweetness and pleasantness of the beverages and palatability of the meal using VAS questionnaires were completed immediately after beverage consumption at 5 min and post-meal at 85 min.

4.3.4.1 Experiment 2

In Experiment 2, the same procedure was followed except for insertion of an indwelling intravenous catheter in the antecubital vein by a registered nurse following the completion of baseline questionnaires. Two baseline blood samples were collected followed by samples collected at 30, 60, 85, 115, and 145 min after the consumption of the pre-meal beverage. Children were asked to remain seated for the duration of the experimental session, and were permitted to engage in a quiet activity.

Whole blood was collected in 3.5 mL BD Vacutainer® SST Plus serum tubes (BD Diagnostics, Franklin Lakes, NJ, USA) coated with silicone and micronized silica particles to accelerate clotting for serum sample collection, and in 2.0 mL BD™ P800 tubes (BD Diagnostics, Franklin Lakes, NJ, USA) containing spray-dried K$_2$EDTA anticoagulant and proprietary additives to prevent the immediate proteolytic breakdown of hormones in plasma samples. The tubes were centrifuged (Allegra X-22R, Beckman Coulter) at 1300 RCF for 10 min at 4°C. Collected serum and plasma were aliquotted into Eppendorf tubes and stored at -70°C for analyses, as previously described [179]. Plasma ghrelin samples were immediately treated with 1N HCl and phenylmethanesulfonyl fluoride (10 mg/mL methanol) per mL of plasma, in order to prevent protein cleavage. Serum concentrations of glucose and insulin, and plasma concentrations of the anorexigenic gut hormones, glucagon-like peptide-1 (GLP-1) and total peptide tyrosine tyrosine (PYY), and the appetite-stimulating gut hormone, total ghrelin, were measured. Serum
concentrations of glucose and insulin were measured at 0 (baseline), 30, 60, 85, 115 and 145 min. Plasma concentrations of GLP-1, PYY and ghrelin were measured at 0, 60, and 115 min post beverage consumption.

Serum glucose was measured using the enzymatic hexokinase method (intra-CV: <5%; inter-CV: <8%; Roche Diagnostics, Indianapolis, IN, USA). Insulin was assessed with an electrochemiluminescence assay “ECLIA” (intra-CV: <3%; inter-CV: <7%; Roche Diagnostics, Indianapolis, IN, USA). These analyses were carried out by the Pathology and Laboratory Medicine Department at Mount Sinai Hospital (Toronto, ON, Canada). Human active GLP-1 (intra-CV: <8%; inter-CV: <5%; #EGLP-35K), total ghrelin (intra-CV: <2%; inter-CV: <8%; #EZGRT-89K), and total PYY (intra-CV: <6%; inter-CV: <8%; #EZHPYYT66K) were analysed with commercially available ELISA kits (Millipore, Billerica, MA, USA).

4.3.5 Statistical Analyses

Statistical analyses were carried out using SAS version 9.2 (SAS Institute Inc., Cary NC, USA). Two-tailed unpaired t-test analysis was used to determine differences in baseline subject characteristics and hormone measures between body weight groups for both experiments. Sex was initially included as a factor affecting FI, glucose and appetite hormones; however, it was removed when there was no effect. Two-way repeated measures analysis of variance (ANOVA) using SAS PROC MIXED procedure were performed to investigate the effects of beverage (Experiment 1: control vs. milk vs. chocolate milk vs. yogurt drink vs. fruit punch; Experiment 2: milk vs. fruit punch), and body weight (NW vs. OW/OB) and their interactions on all dependent measures including FI (pizza kcal), energy intake at the meal (pizza kcal + beverage at the test meal kcal), cumulative energy intake (pre-meal beverage kcal + pizza kcal + beverage at the test meal kcal), caloric compensation, post-meal sweetness and pleasantness of the beverage and pizza meal. Food intake in kcal was expressed in total and corrected for body weight (/kg) because of the large weight differences among children.

For Experiment 2, because concentrations of metabolites reflect the food dose in relation to body weight energy content of the treatments was adjusted to differences in body weight.
between the two groups. Therefore blood glucose, insulin, GLP, PYY and ghrelin concentrations are expressed as units/kcal/kg of treatment consumed.

Caloric compensation was calculated using the following equation: Caloric compensation (%) = \((\text{kcal consumed at meal after water control} - \text{kcal consumed at meal after preload})/\text{kcal in the preload} - \text{kcal in control}\) x 100. A caloric compensation greater than 100% indicated overcompensation and less than 100% indicated low compensation for the beverage energy at the pizza meal.

Average subjective appetite scores, serum concentrations of glucose and insulin and plasma concentrations of appetite hormones were calculated as mean changes from baseline and were grouped into pre- (0–60 min) and post-meal (60–145 min) periods. Pre-meal changes from baseline were calculated from 0 min (immediately before beverage consumption) and post-meal changes from 60 min (before test meal consumption). Three-factor ANOVA analyses were performed to determine the effects of time, beverage, body weight and their interactions on change from baseline average appetite, serum concentrations of glucose and insulin and plasma concentrations of appetite hormones. When an interaction was found, a one-way ANOVA was performed followed by Tukey-Kramer post-hoc test to compare the effect of beverages and body weight groups on serum glucose and insulin, and plasma hormonal responses at each time of measurement.

All results are presented as mean ± standard error of the mean (SEM). Statistical significance was considered at P-value of less than 0.05.

4.4 RESULTS

4.4.1 Participant Characteristics

In Experiment 1 (n = 32, Table 2), sixteen NW (8 females, 8 males), eight OW (4 females, 4 males) and eight OB (4 females, 4 males) children completed all five experimental sessions. However, four children were excluded due to their failure to drink all the beverages or follow the procedures. In Experiment 2 (n = 20, Table 2), ten NW (5 females, 5 males), three OW (1
females, 2 males) and seven OB (4 females, 3 males) children completed the two experimental sessions. Overweight/obese children reported higher restraint scores than NW children (P = 0.006, Table 2) in Experiment 2 but there were no differences in Experiment 1. In both experiments, fat-free mass was similar in OW/OB children to NW children but their higher body weights were accounted for by their 3-fold higher fat mass. Baseline concentrations of serum glucose and insulin, and plasma GLP-1 were similar, but PYY was lower in OW/OB than NW children (Table 3).

4.4.2 Experiment 1

4.4.2.1 Food Intake and Caloric Compensation

Energy (kcal) intake from pizza, energy intake at the meal (pizza + beverage) and cumulative energy intake (pre-meal beverage + pizza + beverage at the test meal) were affected by beverage (P < 0.05, Table 4), but not by body weight or their interaction.

Consumption of pre-meal and mealtime chocolate milk and yogurt drink reduced mean FI by 14% (P < 0.001) and 10% (P = 0.01), respectively, compared to water but not to milk or fruit punch. Conversely, energy intake at the meal was higher after fruit punch (12%, P = 0.03) compared to water. Cumulative energy intake was affected by the caloric content of the beverages, with lower cumulative energy intakes after water compared to all caloric beverages (P < 0.001).

Results were similar when FI was corrected for body weight, with an effect of beverage (P < 0.02, Table 4) and body weight (P < 0.004), but again no beverage by body weight interaction was found. Although treatment effects were not affected by body weight, normal weight children consumed 32% more pizza (18.2 kcal/kg), than OW/OB children (13.4 kcal/kg; P = 0.003; Table 4). Compensation in food consumed for the energy in the beverages was low for all dairy beverages, averaging 65%. However, chocolate milk had greater caloric compensation compared to fruit punch (91% vs. 29%, P < 0.01, Table 4).
4.4.2.2  Subjective Appetite

Pre-meal (0–60 min) average appetite scores were affected by time (P < 0.001), with appetite increasing over the pre-meal period. Average appetite was also affected by beverage (P = 0.004) and a beverage by body weight interaction (P = 0.009). Pre-meal average appetite scores increased more after milk compared to fruit punch (34%; P = 0.03), chocolate milk (44%; P = 0.04), and water (60%; P = 0.006), with no differences compared to the yogurt drink (Figure 1A). The interaction effect can be explained by the strong beverage effect in NW children (P < 0.001, Figure 1B) but not in OW/OB children (Figure 1C).

Post-meal (60–145 min) average appetite scores were affected by time (P < 0.001), beverage (P = 0.001) and an interaction between beverage and body weight (P < 0.001). Compared to the yogurt drink, milk and fruit punch induced greater reductions from baseline averaging 14% (P < 0.001) and 8% (P = 0.04), respectively (Figure 1A). However none of the caloric beverages reduced appetite more than water. The interaction occurred because average appetite was affected by beverage type in NW but not OW/OB children. In NW children, post-meal average appetite scores were reduced more by milk than after chocolate milk or yogurt (P < 0.02, Figure 1B).

4.4.3  Experiment 2

4.4.3.1  Food Intake

Pizza intake, energy intake at the meal and cumulative energy intakes were affected by body weight (P = 0.04), but not by beverage or their interaction. Overweight/obese compared to NW children, consumed 30% more pizza calories (Table 4). When FI was adjusted /kg body weight there was no effect of beverage, body weight or their interaction.

4.4.3.2  Subjective Appetite

Pre-meal average appetite was affected by time (P = 0.03), beverage (P = 0.02) and a beverage by body weight interaction (P < 0.05) but not by body weight, beverage by time or a body weight by time interaction. Pre-meal average appetite increased more after milk compared to
fruit punch (Figure 2A) but only in OW/OB (P = 0.01, Figure 2C). In the post-meal period, average appetite scores reduced over time (P = 0.01), and were affected by beverage (P = 0.03), but not by body weight or beverage by body weight interaction. Irrespective of body weight, milk reduced post-meal average appetite by 8% compared to fruit punch.

4.4.3.3 Serum Glucose Concentrations

Change from baseline blood glucose concentrations (mmol/L), either unadjusted or adjusted for pre-meal beverage intake as kcal/kg body weight, were affected by time (P < 0.001) with values peaking at 30 min after the pre-meal beverage and again at 85 min, immediately after the meal. During the pre-meal (0–60 min) period (Table 5), no differences due to beverages were observed in the unadjusted glucose data. However, glucose concentrations adjusted for the pre-meal beverages expressed as kcal/kg was affected by beverage (P = 0.04), but not by body weight or a beverage by body weight interaction, with lower values after milk than fruit punch (Figure 3A).

Post-meal (60–145 min) glucose concentrations, unadjusted and adjusted (kcal/kg), were affected by body weight (P < 0.04) with higher post-meal glucose in OW/OB than NW children (Figure 3A), but no effect of beverage or interaction.

4.4.3.4 Serum Insulin Concentrations

Insulin concentrations (pmol/L) changes from baseline were affected by time (P < 0.001) but not body weight or beverage, with values peaking at 30 min after the pre-meal beverage and to a greater degree at 85 min immediately after the meal. Pre-meal (0–60 min) insulin, either unadjusted or adjusted for intake (kcal/kg body weight), were not affected by beverage, body weight or their interactions. Post-meal (60–145 min) insulin was affected by body weight (P = 0.006, Figure 3B) with higher serum insulin concentrations in OW/OB compared to NW children when their intakes were adjusted for kcal/kg consumed.
4.4.3.5 Plasma GLP-1 Concentrations

Change from baseline GLP-1 concentrations, either unadjusted or adjusted, were affected by time (P < 0.001) with values peaking at 115 min, 30 min after the meal. There was also a trend for sex (P = 0.058) with greater GLP-1 in female than male participants. During the pre-meal (0–60 min) period, both unadjusted and adjusted GLP-1 were affected by pre-meal beverage (P < 0.05, Figure 4A) with milk resulting in higher GLP-1 response. Post-meal (60–115 min) unadjusted GLP-1 was not affected by beverage, body weight or their interaction, but GLP-1 concentrations adjusted to kcal/kg was affected by body weight (P = 0.01, Figure 4B) with higher post-meal GLP-1 in OW/OB than NW children.

4.4.3.6 Plasma PYY Concentrations

Change from baseline PYY concentrations, unadjusted and adjusted for kcal/kg body weight, was affected by time (P < 0.001) with values peaking at 115 min (30 min after the meal). Pre-meal (0–60 min) PYY, either unadjusted or adjusted, was not affected by beverage, body weight or an interaction. However, post-meal (60–115 min) PYY concentrations, unadjusted and adjusted for kcal/kg body weight were affected by beverage (P < 0.04, Figure 4C), but not body weight (Figure 4D) or a beverage by a body weight interaction. Post-meal PYY response was greater after milk than fruit punch (P < 0.04).

4.4.3.7 Plasma Total Ghrelin Concentrations

Ghrelin concentrations expressed as changes from baseline, either unadjusted or adjusted, were affected by time (P < 0.01) with values decreasing below baseline. Pre-meal (0–60 min) unadjusted and adjusted ghrelin changes from baseline were not affected by body weight, beverage (Figure 4E) and beverage by body weight interaction. Post-meal (60–115 min) adjusted, but not adjusted ghrelin change from baseline was affected by body weight (P = 0.01, Figure 4F) with greater decreases in OW/OB than NW children. No interactions were found.
4.5 DISCUSSION

These studies are the first to examine the effect of consuming common beverages before and with a meal on FI, subjective appetite, glucose, and appetite hormone responses in children. The results of this study do not support the hypothesis that isocaloric dairy products reduce FI and appetite more than fruit punch, a SSB. However, the study provides preliminary data showing that pre-meal milk results in lower pre-meal glucose and more favourable responses in satiety hormones in both NW and OW/OB children. Post-meal metabolic responses were driven primarily by the large amount of food consumed at mealtime, but exaggerated in OW/OB children.

In Experiment 1, beverage composition was a factor affecting FI. However, only yogurt and chocolate milk consumed before and with the meal reduced pizza intake more than water. Milk protein is known to be a component of milk that affects both FI and metabolic regulation [88, 89]. Both of the major protein fractions in milk suppress short-term FI and reduce glycemic response in adults [60, 88] and pre-meal whey protein reduces FI more than isocaloric glucose (1.0 g/kg body weight, 250 mL) at 60 min in normal weight boys [175]. However, milk with the most protein (8 g/250 mL) among treatments did not reduce FI compared to all other beverages. Thus, the response to chocolate milk and yogurt drink but not milk is difficult to explain. One possible explanation is their higher carbohydrate content compared to milk. Independently, sucrose [180, 181] and milk proteins [88, 89, 175, 182] suppress FI, suggesting that the combination of the two macronutrients as found in chocolate milk and yogurt trigger stronger satiety signals than either one alone. However, incomplete mealtime compensation occurred for all caloric beverages resulting in higher cumulative intakes compared with water. These data in children are consistent with previous studies in adults showing that mealtime caloric beverages by-pass caloric regulation [13], supporting recommendations that water may be the preferred mealtime beverage [183].

Although no differences in beverage effects on FI were found between NW and OW/OB groups, as indicated by a lack of interaction between treatment and body weight in either experiment, the OW/OB and NW children differed in the two experiments. Between body weight groups,
total FI was similar in Experiment 1, but OW/OB children ate 30% more in Experiment 2. However in Experiment 1, when corrected to body weight, NW children ate more than the OW/OB while OW/OB children ate similar amounts as NW children in Experiment 2 (Table 4). The inconsistency in FI between groups in Experiments 1 and 2 is difficult to explain. In both body weight groups body composition was similar with a ratio of fat mass:fat-free mass of 1:2. However, it may be partly explained by the differences in their self-reported eating behaviours. A positive relationship is found between restrained eating and being OW/OB [184, 185] with disinhibition in eating patterns, leading to binging that leads to weight gain [184]. In the present studies, OW/OB children in Experiment 2 reported greater restraint than NW children. This was consistent with the fact that while they averaged the same age and height as the OW/OB participants in Experiment 1, they were over 20 kg heavier and may have been exposed to more expressed concerns about their body weight [185].

Similar to previous studies in children, pre-meal average appetite increased over time towards the test meal even after the caloric beverages were consumed [175, 176]. However, in contrast to FI, both pre- and post-meal subjective appetite was affected by treatment, with increases most after milk in only NW children (Figure 1). Overall mean appetite tended to increase more in the OW/OB group with no treatment effect. Post-meal decrease in appetite also showed treatment effects, but again only in NW children, with overall mean decrease of -63 ± 1.3 mm compared with -50 ± 1.2 mm in the OW/OB children. The greater increase in pre-meal and reduction in post-meal average appetite after milk in both experiments was not reflective of mealtime energy intake. There was no correlation between average appetite scores at 60 min with FI, suggesting that subjective appetite measures do not predict FI in children, in contrast to adults [85, 177].

Although 2% milk failed to affect FI compared with fruit punch in either experiment, pre-meal consumption of milk increased satiety hormones, GLP-1 and PYY reflecting the expected effect of consumption of milk proteins [88, 138]. Milk consumed with the meal also increased PYY concentrations, consistent with its response to protein ingestion [89] and with a previous study showing that PYY is one of the potential mediators of milk’s inhibitory effects on hunger and FI [88]. Although FI was not affected, an increase in these hormones is consistent with the reduced
post-meal average appetite scores after milk (Figure 2), which may delay the return of hunger and the next meal-eating occasion [186, 187], supporting the potential of milk to contribute to the body weight control in the long-term.

Higher mealtime FI altered glycemic and appetite hormone responses in OW/OB children more than in NW children. Although baseline glucose/insulin ratios were not different between NW and OW/OB children (Table 3), post-meal insulin concentrations were higher in OW/OB children, but did not improve glucose responses, suggesting that OW/OB children may have reduced insulin sensitivity [188, 189]. However, the insulin response was concordant with higher post-meal GLP-1, which in turn, may have mediated the post-meal reduction in ghrelin through its insulinotropic effects [190]. In contrast, PYY concentrations did not differ between NW and OW/OB children, despite differences in baseline PYY values. This difference between body weight groups indicates that the amount of calories provided by the standardized breakfast (equal for both groups, 300 kcal) was inadequate to elicit the same response in OW/OB compared with NW children [191].

This study adds to the limited literature examining the effects of dairy consumption on FI, and glycemic and appetite hormone response in children, but has several limitations as designed. Although the study was designed to examine the effects of common serving sizes of beverages often consumed by children, interpretation of the metabolic responses is limited for several reasons [192]. First, equal serving sizes of breakfast (300 kcal) and pre-meal and within meal beverages (130 kcal) were provided for both NW and OW/OB groups. Because the latter were approximately 50% heavier, impact of the beverages on them would be expected to be less, thus leading to a false negative effect in the OW/OB group. For example the higher body weight of the OW/OB group may explain their lack of differences in the response to milk and fruit punch in contrast to NW children. As well, it can be expected that the lower intakes in proportion to body weight would also reduce responses in blood concentrations of metabolic measures. For this reason the measures were adjusted to express concentrations relative to kcal/kg consumed. Second, Experiment 2 had a small sample size and had unbalanced body weight and sex groups. Although differences were detected in glucose and appetite hormones, it was inadequate to
properly assess FI. Third, not all of the beverages were included in Experiment 2, which makes it more difficult to explain the results of Experiment 1. Finally, since only a fixed amount of the beverage was given, the OW/OB children may have required more fluids to consume with the meal to maintain fluid balance [34]. These shortcomings can be addressed in future studies.

In conclusion, fluid dairy products do not reduce FI and subjective appetite more than other caloric beverages, but milk results in lower pre-meal glucose and more favourable responses in satiety hormones in NW and OW/OB children.
**TABLE 1.** Energy and macronutrient composition of beverages and test meal

<table>
<thead>
<tr>
<th>Beverages</th>
<th>2% Milk(^2)</th>
<th>1% Chocolate Milk(^3)</th>
<th>1.5% Yogurt Drink(^3)</th>
<th>Fruit Punch(^2)</th>
<th>Water(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>255.5</td>
<td>202.5</td>
<td>177.5</td>
<td>241</td>
<td>250</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>250</td>
<td>191</td>
<td>173</td>
<td>232</td>
<td>250</td>
</tr>
<tr>
<td>Water Chaser (mL)</td>
<td>0</td>
<td>59</td>
<td>77</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Total Volume (mL)</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>130</td>
<td>0</td>
</tr>
<tr>
<td>Energy density (kcal/g)</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>5.2</td>
<td>1.9</td>
<td>2.4</td>
<td>&lt;0.1</td>
<td>0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>8.2</td>
<td>5.7</td>
<td>4.1</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>12.7</td>
<td>22.8</td>
<td>23.2</td>
<td>32.1</td>
<td>0</td>
</tr>
<tr>
<td>Total Sugars (g)</td>
<td>11.2</td>
<td>18.8</td>
<td>17.9</td>
<td>25.8</td>
<td>0</td>
</tr>
<tr>
<td>Fructose (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14.2</td>
<td>0</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.8</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>0</td>
<td>11.2</td>
<td>13.4</td>
<td>1.7</td>
<td>0</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>11.2</td>
<td>7.6</td>
<td>4.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>109.2</td>
<td>154</td>
<td>59.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Calcium</td>
<td>330</td>
<td>190</td>
<td>170</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^1\) Data provided by Maxxam Analytics, Mississauga, ON, Canada
\(^2\) Beverages used in Experiment 1 and 2.
\(^3\) Beverages used in Experiment 1.
TABLE 2. Baseline characteristics of participants

| Characteristics | Experiment 1 | | | Experiment 2 | | |
|-----------------|-------------|---|---|-------------|---|
|                 | Normal (n = 16) | Overweight/Obese (n = 16) | P | Normal (n = 10) | Overweight/Obese (n = 10) | P |
| Age (y)         | 12.0 ± 0.3 | 12.2 ± 0.4 | NS | 12.4 ± 0.7 | 12.0 ± 0.5 | NS |
| Female:Male     | 8:8        | 8:8        | -  | 5:5         | 5:5         | -  |
| Body weight (kg)| 43.1 ± 2.0 | 59.3 ± 3.0* | 0.0001 | 45.7 ± 4.3 | 69.7 ± 6.3* | 0.006 |
| Height (cm)     | 155.5 ± 2.1 | 155.4 ± 2.3 | NS | 155.7 ± 5.5 | 155.2 ± 3.8 | NS |
| BMI percentile  | 46.7 ± 7.0 | 96.2 ± 0.7* | < 0.0001 | 50.8 ± 9.5 | 98.1 ± 0.9* | 0.0007 |
| Fat mass\(^2\) (kg) | 6.8 ± 0.8 | 20.7 ± 2.0* | < 0.0001 | 7.5 ± 1.5 | 24.9 ± 4.5* | 0.003 |
| Fat-free mass\(^2\) (kg) | 36.4 ± 1.8 | 38.6 ± 1.6 | NS | 38.2 ± 3.7 | 44.9 ± 3.6 | NS |
| Restrained eating\(^3\) | 1.5 ± 0.1 | 1.7 ± 0.1 | NS | 1.4 ± 0.1 | 1.8 ± 0.1* | 0.006 |

\(^1\) Data are means ± SEM. n = 32. * Significantly different from normal weight P < 0.001 (unpaired t-test).
\(^2\) Body composition determined by Bioelectrical Impedance Analysis based on the Horlick equation [173].
\(^3\) Restrained eating was measured with the Dutch Eating Behaviour Questionnaire [174].
**TABLE 3.** Experiment 2: Baseline blood parameters\(^1\) of participants\(^2\)

<table>
<thead>
<tr>
<th>Blood parameters(^1)</th>
<th>Normal ((n = 10))</th>
<th>Overweight/Obese ((n = 10))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.8 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>88.4 ± 9.2</td>
<td>106.2 ± 14.9</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose:Insulin Ratio</td>
<td>0.07 ± 0.1</td>
<td>0.07 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>GLP-1 (pg/mL)</td>
<td>4.9 ± 0.9</td>
<td>5.5 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>PYY (pg/mL)</td>
<td>157.4 ± 10.0</td>
<td>86.2 ± 8.9*</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total Ghrelin (pg/mL)</td>
<td>517.5 ± 36.2</td>
<td>581.7 ± 48.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^1\) Blood parameters are mean of both treatment sessions.
\(^2\) Data are means ± SEM. \(n = 20.\) * Significantly different from normal weight \(P < 0.001\) unpaired t-test.\)
TABLE 4. Effect of beverages on pizza intake, energy intake at the meal, cumulative energy intake and caloric compensation

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Energy intake (kcal)</th>
<th>Energy intake (kcal/kg body weight)</th>
<th>Caloric compensation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pizza Intake</td>
<td>Meal</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>NW</td>
<td>OW/OB</td>
</tr>
<tr>
<td>2% Milk</td>
<td>772.0 ± 47.8ab</td>
<td>749.4 ± 68.7b</td>
<td>793.1 ± 47.8ab</td>
</tr>
<tr>
<td>Chocolate Milk</td>
<td>720.8 ± 43.1a</td>
<td>746.5 ± 65.0a</td>
<td>693.4 ± 57.3a</td>
</tr>
<tr>
<td>Yogurt Milk</td>
<td>751.4 ± 43.2a</td>
<td>704.1 ± 74.2</td>
<td>795.7 ± 43.3a</td>
</tr>
<tr>
<td>Fruit Punch</td>
<td>799.3 ± 49.2b</td>
<td>789.0 ± 77.2</td>
<td>809.0 ± 43.3a</td>
</tr>
<tr>
<td>Water (control)</td>
<td>828.3 ± 40.9b</td>
<td>872.0 ± 74.2</td>
<td>778.3 ± 49.3a</td>
</tr>
<tr>
<td>P</td>
<td>0.0005</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Marginal values</td>
<td>774.0 ± 20.1</td>
<td>773.1 ± 27.4</td>
<td>774.8 ± 29.6</td>
</tr>
</tbody>
</table>

| 2% Milk        | 1001.2 ± 72.6        | 811.4 ± 88.2 | 1172.1 ± 72.6 | 1131.7 ± 262.2 | 18.7 ± 1.5 | 19.6 ± 2.4 | 17.8 ± 1.9 | NS |
| Fruit Punch    | 1014.6 ± 71.8        | 889.4 ± 99.3 | 1139.8 ± 71.8 | 1144.8 ± 1274.9 | 18.5 ± 1.2 | 19.9 ± 1.5 | 17.2 ± 1.7 | NS |
| P              | NS                   | NS            | NS         | NS           | NS        | NS        | NS           | NS |
| Marginal values| 1008.1 ± 50.4         | 852.4 ± 61.0 | 1155.9 ± 50.4 | 1138.4 ± 1268.7 | 18.6 ± 0.9 | 19.7 ± 1.4 | 17.5 ± 1.2 | NS |

1 Data are means ± SEM. (n = 32 in experiment 1 and n = 20 in experiment 2). One-factor ANOVA analysis for energy intake, energy intake adjusted for body weight and caloric compensation. Values in the same column with different superscript letters are significantly different, P < 0.05 (beverage effect using proc mixed, Tukey-Kramer post-hoc test). NW (normal weight), OW/OB (overweight/obese).
2 Pizza only consumed at 60 min
3 Beverage plus pizza consumed at 60 min
4 Pre-meal beverage consumed at baseline plus beverage and pizza consumed at 60 min
5 Caloric compensation = [(kcal consumed at meal after water control – kcal consumed at meal after preload)/kcal in the preload – kcal in control] x 100.
TABLE 5. Experiment 2: Mean changes from baseline pre- and post-meal blood glucose and insulin, and appetite hormone responses unadjusted and adjusted for pre-meal beverage kcal/kg body weight

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Normal Weight (n = 10)</th>
<th>Overweight/Obese (n = 10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2% Milk</td>
<td>Fruit Punch</td>
<td>2% Milk</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>0.2 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Post-meal</td>
<td>0.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adjusted Glucose (mmol/L/kcal/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>0.03 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post-meal</td>
<td>0.1 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>36.2 ± 18.3</td>
<td>73.0 ± 28.0</td>
<td>53.6 ± 29.9</td>
</tr>
<tr>
<td>Post-meal</td>
<td>329.9 ± 72.8</td>
<td>264.4 ± 54.5</td>
<td>348.8 ± 57.5</td>
</tr>
<tr>
<td>Adjusted Insulin (pmol/L/kcal/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>11.4 ± 6.0</td>
<td>28.6 ± 11.7</td>
<td>24.0 ± 14.4</td>
</tr>
<tr>
<td>Post-meal</td>
<td>124.0 ± 30.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.0 ± 24.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>181.1 ± 30.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GLP-1 (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>0.2 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.7 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post-meal</td>
<td>3.7 ± 0.3</td>
<td>2.7 ± 0.8</td>
<td>4.6 ± 1.03</td>
</tr>
<tr>
<td>Adjusted GLP-1 (pg/mL/kcal/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>0.1 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.2 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post-meal</td>
<td>1.2 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PYY (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>-13.4 ± 9.4</td>
<td>-7.7 ± 7.0</td>
<td>10.9 ± 5.0</td>
</tr>
<tr>
<td>Post-meal</td>
<td>62.9 ± 10.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.5 ± 12.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.3 ± 9.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adjusted PYY (pg/mL/kcal/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>-3.4 ± 2.7</td>
<td>-2.6 ± 2.8</td>
<td>5.2 ± 2.7</td>
</tr>
<tr>
<td>Post-meal</td>
<td>20.7 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.0 ± 5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.4 ± 6.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ghrelin (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>-48.6 ± 13.1</td>
<td>-69.0 ± 31.2</td>
<td>-11.5 ± 25.1</td>
</tr>
<tr>
<td>Post-meal</td>
<td>-55.2 ± 21.6</td>
<td>-71.4 ± 18.4</td>
<td>-153.8 ± 37.8</td>
</tr>
<tr>
<td>Adjusted Ghrelin (pg/mL/kcal/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-meal</td>
<td>-16.8 ± 4.5</td>
<td>-23.0 ± 11.0</td>
<td>-4.4 ± 10.7</td>
</tr>
<tr>
<td>Post-meal</td>
<td>-19.7 ± 7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-27.1 ± 7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-72.1 ± 11.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data are means ± SEM; (n = 20). Two-factor ANOVA analysis for pre- and post-meal mean changes in blood concentrations, unadjusted and adjusted (expressed unit/kcal/kg body weight for pre-meal beverage intake) was performed with beverage and body weight as independent factors. Values in the same row with different superscript letters are significantly different (P < 0.05).

<sup>2</sup>Pre-meal values are mean changes from 0–60 min before the test meal.

<sup>3</sup>Post-meal values are means changes after the test meal and calculated from 60–145 min for glucose and insulin and 60–115 min for GLP-1, PYY and ghrelin.
FIGURE 1. Experiment 1: Subjective appetite changes from baseline pre- (0–60 min) and post-meal (60–145 min) of (A) all, (B) normal weight and (C) overweight/obese children. Means with different letters are significantly different (three-factor repeated-measures ANOVA followed by Tukey-Kramer post-hoc test was used to compare the effect of beverage, \( P < 0.004 \), interaction between beverage and body weight, \( P < 0.01 \) and time, \( P < 0.001 \). All values are mean ± SEM; \( n = 32 \) all children; \( n = 16 \) normal weight; \( n = 16 \) overweight/obese.
FIGURE 2. Experiment 2: Subjective appetite changes from baseline pre- (0–60 min) and post-meal (60–145 min) of (A) all, (B) normal weight and (C) overweight/obese children. * Significantly different from fruit punch (three-factor repeated-measures ANOVA followed by Tukey-Kramer post-hoc test was used to compare the effect of beverage, \( P < 0.02 \), interaction between beverage and body weight, \( P < 0.05 \) and time, \( P < 0.001 \). All values are mean ± SEM; \( n = 20 \) all children; \( n = 10 \) normal weight; \( n = 10 \) overweight/obese.
FIGURE 3. Experiment 2: Serum (A) blood glucose and (B) insulin changes from baseline, adjusted for kcal/kg, pre- and post-meal. *Significantly different from fruit punch. # Significantly different from overweight/obese. Two-factor ANOVA (Pre-meal: Beverage, $P < 0.05$, Body weight, $P > 0.05$, Beverage*Body weight, $P > 0.05$; Post-meal: Beverage, $P > 0.05$, Body weight, $P < 0.05$, Beverage*Body weight, $P > 0.05$). All values are means ± SEM; $n = 20$. 
FIGURE 4. Experiment 2: Plasma (A, B) GLP-1, (C, D) PYY and (E, F) ghrelin changes from baseline adjusted for pre-treatment intake (kcal/kg). *Significantly different from fruit punch. # Significantly different from overweight/obese. Two-way ANOVA (beverages and time or body weight and time), Tukey-Kramer post hoc test, $P < 0.05$. All values are means ± SEM; $n = 20$. 
Chapter 5
General Discussion & Future Directions

This research provides evidence that milk consumption may have positive physiological outcomes on glycemic and appetite hormone control in children. However, more short- and long-term experimental studies with blood sampling are needed to explore body composition, pubertal development and their interactions on the physiological aspects of appetite control to elucidate the effects of fluid dairy in children. Furthermore, determining the quantity of milk required to elicit the positive effects of milk intake can assist with dairy recommendations in children and adolescents. In addition, the present study adds to the limited research in understanding environmental and physiological factors affecting food intake (FI), behaviour and nutritional health in children [192].

Long-term experimental studies in children show that calcium intake associates positively with reduction of body fat [167], but increased dairy does not consistently show this [193]. Few studies investigated the role of dairy or any food on the physiological mechanisms that govern FI in children. In contrast, research has overwhelmingly been focused on environmental factors, including energy dense, sweet and savoury foods and lack of activity as the causative factor of obesity in children [194]. However, it has yet to be determined whether obesity develops in susceptible individuals because physiological mechanisms of FI control are compromised first or if these are simply overridden by the environment and become compromised [195]. Understanding this relationship requires the identification of the primary cause of excessive energy intake and energy imbalance, and determine if the causes are primarily physiological or environmental.

Non-food, food-related and mealtime factors may each contribute to how much energy is consumed at a meal and can also operate together to impact FI. In adults, non-food related factors such as reduced physical activity, alcohol consumption, smoking, socioeconomic status and mental illness negatively impact body weight. Food-related environmental factors relates to the way food is provided or presented, such as its salience, variety, package or portion size, and palatability. As well, the mealtime environment and the amount eaten is dependent on how food
is obtained, the eating atmosphere, the social interactions that occur and the distractions that 
may be present during the meal [196]. Compared with studies in adults, research with children 
from 7–17 years is very limited, leading to the unfortunate assumption that what applies to 
adults applies to children and can be used to develop dietary guidance for children. Dietary 
advice can oftentimes be misleading in both adults and children as new research emerges that 
disproves previous held beliefs. As such, new approaches are needed to help understand the 
complexities in the roles of environment and physiology in FI.

The present study also shows the value of short-term studies in elucidating interaction between 
the environment and physiology. There are several reasons to conduct short-term studies. First, 
the effects of each of the macronutrients on short-term satiety signals are unique and cannot be 
easily identified based on meals or habitual diets. Second, excess energy intake is ultimately 
derived from failure to respond to short-term signals arising from food consumption and to 
delayed termination of eating (satiation) at meals. Third, these studies can be useful to identify 
strategies to optimize satiety signals arising from a formulated snack or pre-meal supplements to 
reduce later energy intake and to formulate meals that lead to earlier satiation. Fourth, because 
Health Canada has approved labelling foods with satiety claims, it is important to makes claims 
that are scientifically sound based on an understanding of the relationships between satiety in 
response to a food or beverage and later energy intake. Satiety claims based on adult testing may 
not apply to children. Fifth, this knowledge is required prior to designing effective strategies for 
managing over-eating by children within real life situations.

Food intake regulation is primarily a physiologic process that is a powerful driver aimed to 
ensure that energy intakes meet requirements [197]. The variables proposed above as causal 
factors of obesity may contribute to energy intake exceeding requirements, but the failure of 
food and meals to provide satiation can be argued to be a more significant factor. For example, 
walking for 30 min leads to only 100 kcal of expenditure [198, 199], which can be easily 
overridden by choosing to hydrate with a sweetened beverage such as a soft-drink or cup of 
juice (140 kcal). Environmental factors that diminish feelings of satiety and satiation may be 
more plausible causes of energy imbalance than lack of activity or the food per se. In children,
this energy imbalance has been associated with physiological factors including sex, age, pubertal stage, body fatness and fitness and a long list of environmental factors, including socioeconomic status, ethnicity, parenting, eating out, food composition, rapid eating, snacking, quick service restaurants, stress, television viewing, lack of exercise, peer group and many more [200]. Many public health actions have been undertaken or proposed based on these factors, yet obesity continues to increase.

Thus, while this study pointed to several short-term experiments, longer-term studies that allow examination of environmental influences on beverage consumption are needed. Several short-term randomized studies based on repeated measures designs could be conducted to add to an understanding of the role of beverages in FI regulation. First, although the study was designed to examine the effects of common serving sizes of beverages often consumed by children, the higher body weight of the OW/OB group may explain their lack of differences in the response to milk and fruit punch in contrast to NW children. Thus an interpretation of the metabolic and food intake responses would be enhanced by providing on the basis of body weight and provide a greater variety of beverage treatments that differ in composition. Second, an experiment should be designed with an adequate sample size to measure both metabolic responses as well as subjective appetite and food intake in the same experiment. This may allow a determination of the most relevant biomarkers of satiety in both OW/OB and NW children. To further examine the relationship between beverage and food calories both could be provide ad libitum, as often occurs in home or restaurant environments. Finally, a more holistic study that may provide solution to advice on beverage consumption would be a well-designed longitudinal study that follows children through critical stages of their development and provide clarity on the key environmental and physiological factors that interact to serve as risk factors for childhood and hence, adult obesity.
References


42. Hu FB. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. Obes Rev 2013;14:606-19.


179. Patel BP, Anderson GH, Vien S, Bellissimo N, McCrindle BW, Hamilton JK. Obesity, sex and pubertal status affect appetite hormone responses to a mixed glucose and whey


Appendices

Appendix 1. Sample Size Calculation

Sample size estimation when testing for the mean of a normal distribution (two-sided alternative). For a within subject design, the equation is:

\[ n = \left( \frac{Z_{1-\alpha/2} + Z_{1-\beta}}{\sigma/\Delta} \right)^2 \]

\( \alpha = 0.05, \) probability of Type I error

\( \beta = 0.20, \) probability of Type II error

\( Z_{0.975} = 1.96 \)

\( Z_{0.80} = 0.84 \)

\( \sigma = 269 \)

\( \Delta = 212 \text{ kcal} \)

\( n = 24 \)

Values were taken from previous beverage studies done in children of the same age [175]. \( \sigma \) represents standard deviation, \( \Delta \) represents the minimal difference in food intake observed between a glucose beverage and water control, \( n \) is the number of subjects required.
Appendix 2. Experimental Protocol

Experiment #1
- Fasting for 2 hours
- Standard Breakfast
- Ad libitum Food Intake
- Blood sampling for serum glucose and insulin
- Blood sampling for plasma appetite hormones

Experiment #2
- Blood sampling for subjective appetite

Visual Analogue Scales for subjective appetite
Appendix 3. Beverage & Pizza Composition

7.3.1 Manufacturer Beverage Composition Data

<table>
<thead>
<tr>
<th>Energy &amp; Macronutrients per one serving</th>
<th>Cow's Milk (2%) (Beatrice)</th>
<th>Chocolate Milk (1%) (Beatrice)</th>
<th>Yogurt Drink (Strawberry, YOP - Yoplait)</th>
<th>Fruit Punch (Tropical Rhythms)</th>
<th>Water (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>250</td>
<td>250</td>
<td>200</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>130</td>
<td>170</td>
<td>150</td>
<td>140</td>
<td>0</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>5.0</td>
<td>2.5</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Saturated (g)</td>
<td>3.0</td>
<td>1.5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trans (g)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>20</td>
<td>28</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9.0</td>
<td>9</td>
<td>5</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>12</td>
<td>28</td>
<td>26</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>12</td>
<td>28</td>
<td>26</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>120</td>
<td>220</td>
<td>80</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

7.3.2 Maxxam Analytics Beverage Composition Data

<table>
<thead>
<tr>
<th>Energy &amp; Macronutrients per one treatment</th>
<th>Cow's Milk (2%) (Beatrice)</th>
<th>Chocolate Milk (1%) (Beatrice)</th>
<th>Yogurt Drink (Strawberry, YOP - Yoplait)</th>
<th>Fruit Punch (Tropical Rhythms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>260</td>
<td>200</td>
<td>175.68</td>
<td>245.28</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td><strong>130</strong></td>
<td><strong>130</strong></td>
<td><strong>130</strong></td>
<td><strong>130</strong></td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>5.17</td>
<td>1.85</td>
<td>2.40</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>8.24</td>
<td>5.72</td>
<td>4.13</td>
<td>0.34</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>12.74</td>
<td>22.8</td>
<td>23.19</td>
<td>32.13</td>
</tr>
<tr>
<td>Total Sugars (g)</td>
<td>11.18</td>
<td>18.8</td>
<td>17.92</td>
<td>25.75</td>
</tr>
<tr>
<td>Fructose (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14.23</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9.81</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>0</td>
<td>11.2</td>
<td>13.35</td>
<td>1.72</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>11.18</td>
<td>7.6</td>
<td>4.57</td>
<td>0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>109.2</td>
<td>154</td>
<td>59.73</td>
<td>0</td>
</tr>
</tbody>
</table>

(Maxxam Analytics, Mississauga, ON, Canada)
7.3.3 Pizza composition data from manufacturer

<table>
<thead>
<tr>
<th>Energy &amp; Macronutrients per one pizza</th>
<th>Pepperoni</th>
<th>Three Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>87</td>
<td>81</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Saturated (g)</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Trans (g)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>400</td>
<td>360</td>
</tr>
</tbody>
</table>

(McCain Foods Ltd, Florenceville, NB, Canada)
Appendix 4. Recruitment Advertisements & Letters

7.4.1 Metro Newspaper Ad

University of Toronto
Department of Nutritional Sciences

ATTN: PARENTS OF CHILDREN AGED 9-14 YEARS

We are conducting a research study to learn more about milk products in child nutrition.

REQUIREMENTS: 9-14 year old boys & girls, Healthy, have been born at term and not be taking medication

INVOLVES: screening with the information session + 5 weekend sessions with clinical tests.
Children will be asked to drink or eat common snacks.
Pizza lunch will be provided.

As a reward for taking part:

i) The child will receive a $20 movie pass for each session WITHOUT clinical tests and/or

ii) The child will receive a $50 choice of movie pass or gift certificate to the bookstore for each session WITH clinical tests.

Plus $12 for parents for travel reimbursement

Please contact us at: 416-433-9014
Research Study: How do snacks affect our appetite?
Principal Investigator: Dr Jill Hamilton

Study Description: Researchers at SickKids and University of Toronto are conducting a study looking at the effect of milk on food intake and blood glucose levels in children. Through this study, we hope to create more effective strategies and health care programs to help children reach and maintain a healthy weight.

The study involves a total of 7 visits:
• 5 at University of Toronto with no clinical tests
• 2 at the Hospital for Sick Children with clinical tests - blood sample collection
• At all visits your child will consume a common drink or snack followed by a pizza lunch. Your child will also complete some questionnaires related to their hunger

You may choose to do all 7 visits, or the University of Toronto visits.

Recruitment Information: We are inviting both healthy weight and overweight children and adolescents between the ages of 9-14 years to participate. After each visit we will provide you with a thank you gift and you will be reimbursed for parking/ travel.

Contact Name: Munaza Jamil
Telephone: 416.813.7654 x28363
Email: munaza.jamil@sickkids.ca
Do you want to help us learn more about the effects of milk on childhood obesity?

We are looking for volunteers boys and girls age 9 to 14 years

What will be required?

Screening with the information session
+ 5 weekend sessions at University of Toronto without clinical tests
+ 2 weekend sessions at The Hospital for Sick Children with clinical tests
Children will be asked to drink or eat common snacks.
Pizza lunch will be provided!

You may choose to do all 7 visits, or select to do only the University of Toronto visits.

You will be given a gift in recognition of your participation, and also reimbursed for travel expenses

For more information please contact:
Munaza 416-813-7654 ex 28363
email: munaza.jamil@sickkids.ca
7.4.4 Experiment 1: U of T Recruitment Letter

Department of Nutritional Sciences
FitzGerald Building, 150 College Street, 3rd Floor
Toronto, ON M5S 3E2
CANADA

The Effect of Fluid Dairy Products Consumed Before and Within a Mixed Meal on Blood Glucose, Food Intake and Satiety in Normal Weight and Overweight/Obese Children (Sessions WITHOUT clinical testing)

Recruitment Letter for Parents

Dear Parent

The University of Toronto is leading a team of researchers investigating the effects of milk products on energy intake regulation in children and young adolescents. The ultimate goal of this research is to find ways to address the problems of overeating and obesity that are becoming a concern among those people involved in the long term health of Canadians.

We are asking the parents of girls and boys 9 - 14 years old to allow their daughter/son to take part in a research study. On five separate mornings your child will consume a drink followed by a pizza lunch 60 minutes later. In addition, your child will be asked to complete questionnaires. The study will take place on five weekend, holiday or summer mornings at the FitzGerald Building, Department of Nutritional Sciences (150 College Street, Room 329).

There are criteria for participation that you need to be aware of, the child must:

• be between 9 to 14 years of age, and
• be healthy, and have been born at term, and
• not be taking medications.

As a reward for taking part, at each session the child will be given a movie pass ($20 gift certificate). In addition parents will be reimbursed for travel/parking expenses ($12).

This study has been fully approved by the Health Sciences Research Ethics Board.

If you would like your son/daughter to participate, or to get further information beyond that provided in this letter, please contact Ms. Shirley Vien (416) 978-4153, Dr. Bohdan Luhovyy (902) 457-6256 or Dr. G. Harvey Anderson, Professor (416) 978-1832 at the University of Toronto (Department of Nutritional Sciences).

Thank you for your support in this important research.

Sincerely,
Drs. Harvey Anderson and Bohdan Luhovyy
7.4.5 Experiment 2: U of T Recruitment Letter

Dear Parent

The University of Toronto is leading a team of researchers investigating the effects of milk products on energy intake regulation in children and young adolescents. The ultimate goal of this research is to find ways to address the problems of overeating and obesity that are becoming a concern among those people involved in the long term health of Canadians.

We are asking the parents of girls and boys 9 - 14 years old to allow their daughter/son to take part in a research study. On two separate mornings your child will consume a drink followed by a pizza lunch 60 minutes later. In addition, blood will be sampled throughout the morning and your child will be asked to complete questionnaires. The study will take place on two weekend, holiday or summer mornings at The Hospital for Sick Children (555 University Avenue, 5th floor – Physiological Research Unit).

There are criteria for participation that you need to be aware of, the child must:
• be between 9 to 14 years of age, and
• be healthy, and have been born at term, and
• not be taking medications.

As a reward for taking part, at each session the child will be given a movie pass or gift cards to the bookstore ($50 gift certificate). In addition parents will be reimbursed for travel/parking expenses ($12).

This study has been fully approved by the Health Sciences Research Ethics Board.

If you would like your son/daughter to participate, or to get further information beyond that provided in this letter, please contact Ms. Shirley Vien (416) 978-4153, Dr. Bohdan Luhovyy (902) 457-6256 or Dr. G. Harvey Anderson, Professor (416) 978-1832 at the University of Toronto (Department of Nutritional Sciences).

Thank you for your support in this important research.

Sincerely,
Drs. Harvey Anderson and Bohdan Luhovyy
How Do Snacks Affect our Appetite?

Researchers at SickKids and the University of Toronto are conducting a study to explore the effects of milk products on energy intake regulation in children and young adolescents. From this research, we hope to create more effective strategies to help youth who are struggling with overweight and obesity.

We are asking girls and boys aged 9-14 years to take part in a research study. The study will be conducted at two locations, The University of Toronto and The Hospital for Sick Children.

Five morning visits will be conducted at the University of Toronto; your child will consume a drink or snack followed by a pizza lunch. In addition your child will be asked to complete some questionnaires related to their hunger.

The two visits at The Hospital for Sick Children will be similar except we will also collect blood samples to determine your child’s blood sugar and insulin levels.

You may choose to do all 7 visits, or select to do only the University of Toronto visits.

In total, each visit will last approximately 4 hours with your child arriving at 9:00 am and leaving by 1:00 pm. Participating in this study will require approximately 28 total hours of time over a 7 week period.

In order to participate in the study, the child must:
• be between 9 to 14 years of age, and
• be healthy, and have been born at term, and
• not be taking medications

As a reward for taking part, at each University of Toronto session the child will be given a free movie pass ($20). At each SickKids session, the child can choose between a movie pass or bookstore gift certificate ($50). In addition, parents will be reimbursed for travel/parking expenses ($12).

Please contact us if you would like to hear more about this study.

Sincerely,

Dr. Jill Hamilton
Pediatric Endocrinologist
416-813-7595

Dr. G. Harvey Anderson
Co-Investigator
416-978-1832

Munaza Jamil
Study Co-ordinator
416-813-7654 ext.28363
Appendix 5. Consent Forms

7.5.1 Experiment 1: Parent’s Consent Form

FACULTY OF MEDICINE
University of Toronto

Department of Nutritional Sciences
FitzGerald Building, 150 College Street, 3rd Floor
Toronto, ON M5S 3E2
CANADA

The Effect of Fluid Dairy Products Consumed Before and Within a Mixed Meal on Blood Glucose, Food Intake and Satiety in Normal Weight and Overweight/Obese Children

(Sessions WITHOUT clinical testing)

Study Information Sheet and Parent’s Consent Form

Investigators: Dr. G. Harvey Anderson, Principle Investigator
Department of Nutritional Sciences, University of Toronto
Phone: (416) 978-1832
Email: harvery.anderson@utoronto.ca

Dr. Bohdan Luhovyy, Assistant Professor
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: (902) 457-6256
Email: bohdan.luhovyy@msvu.ca

Dr. Jill Hamilton, Associate Professor
Department of Paediatrics, University of Toronto
Phone: (416) 813-5115
Email: jill.hamilton@sickkids.ca

Dr. Nick Bellissimo, Assistant Professor
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: (902) 457-6295
Email: nick.bellissimo@msvu.ca

Ms. Barkha Patel, Ph.D. Candidate
Department of Nutritional Science, University of Toronto
Phone: (416) 978-4153
Email: barkha.patel@utoronto.ca
Ms. Shirley Vien, Project Coordinator  
Department of Nutritional Science, University of Toronto  
Phone: (416) 978-4153  
Email: shirley.vien@utoronto.ca

Mr. Chris Smith, Lab Manager  
Department of Nutritional Science, University of Toronto  
Phone: (416) 978-6894  
Email: chris.smith@utoronto.ca

Ms. Shari Berengut, Research Assistant  
Department of Nutritional Science, University of Toronto  
Phone: (416) 978-6894  
Email: shari.berengut@gmail.com

Purpose of Research:

The purpose of this study is to determine the effects of dairy drinks on food intake and blood sugar regulation in 9-14 year-old children. This experiment is being conducted through the Department of Nutritional Sciences at the University of Toronto by Dr. G. Harvey Anderson (principal investigator). Your son/daughter will be required to attend one screening session to measure physiological parameters and five experimental sessions conducted over a 5-week period for a total of 6 visits to the University of Toronto campus. Experimental sessions will last a maximum of 3 hours.

The purpose of our research is to develop an understanding of factors affecting the control of food intake and blood glucose in children. Knowing the determinants of the regulation of food intake and blood glucose in children will allow us to develop strategies and recommendations for the prevention of obesity and diabetes.

Procedure:

Screening:

For those parents who express interest in having their son/daughter participate, some information about the child will be requested by telephone. If the child was born at term, is healthy and does not receive any medications, a screening session will be arranged.

During the screening session, the researcher will explain the full details of the study. Parents that give consent to have their son/daughter participate will sign a consent form. The parent will receive copies of the consent form and of the study information sheet. If the child wishes to participate and signs a children’s assent form, his/her weight, height, and body fat using painless techniques, will be measured.
The boys and girls will then be asked to rank their preference for pizza that will be served as the lunch meal at each session.

The children’s physical activity and eating habits will be assessed with Physical Activity Questionnaire and the Dutch Eating Behaviour Questionnaire.

Menstrual Cycle Questionnaire:

Girls will be asked to complete a questionnaire about their menstrual cycle. This information is collected because studies have shown that energy intake and appetite change across the menstrual cycle.

Tanner Staging

To assess the effect of pubertal stage on food intake in children, a questionnaire relating to puberty and 3 cartoon images will be administered to the children in lieu of an examination. The children will be asked to circle the number on the side of the picture that best represents them. Tanner stages are scales that assess physical development in children and adolescents, based on external primary and secondary sex characteristics, such as the size of the breasts, genitalia, and development of pubic hair. The way in which appetite is regulated is related to where children are in their pubertal development. In order to assess pubertal stage, the children will be asked to complete a questionnaire about puberty and changes in their bodies. Depending on the sex of the child, the children will be presented with cartoon pictograms of different stages of physical/sexual development (e.g., breast size, pubic hair, genitalia) and the children will be asked to pick the picture that best represents their stage of puberty. These pictograms have been used extensively in children. If for any reason the children are not willing to participate, they have the option of asking their parents to answer the questionnaire and select the pictograms for them. The children may decline the pubertal staging if they wish. Parents are welcome to discuss the reasons for including Tanner stages as part of the study or any comment or concerns with Dr. Jill Hamilton at jill.hamilton@sickkids.ca

Body Composition Assessment:

Two methods will be used to estimate the amount of muscle and fat tissue in your child’s body.

**Skinfolds:** The skinfold thickness technique is performed by pinching the skin between the thumb and forefinger and placing calipers on the fold measuring the width of thickness of the two layers of skin and subcutaneous fat underneath. The assumptions underlying the rationale of measuring skinfold thickness are that skinfold thickness is an adequate measure of subcutaneous fat (fat under the skin) and that there is a defined relationship between subcutaneous fat and total body fat.

**Bioelectrical Impedance Analysis:** Bioelectrical impedance analysis (BIA), a recently developed technique for measuring body fat content in both adults and children, is simple and painless and is an effective method for measuring body fat in children. BIA is based on measurement of electrical resistance in the body to a tiny current (that the subject cannot feel). The principle of
BIA lies in that muscle mass in the body is a better conductor of electricity than fat, which contains lesser amounts of water and electrolytes.

Appetite, Food Intake and Blood Sugar Assessment:

The boys/girls who participate in this study, will be requested to go to the FitzGerald Building, Department of Nutritional Sciences, University of Toronto, for five individual morning sessions. These sessions will be held on weekends, over five weeks. The children will be brought to the laboratory and returned home by parents only.

On each of the five test days, the children will have a standardized breakfast of cereal, milk and juice at home, either at 8:00 am or 9:00 am (the time will be consistent for each child). The children will arrive at the FitzGerald Building, either at 10:00 am or 11:00 am (but consistent throughout for each child).

Children will fast for 12 hours before breakfast and after breakfast until their arrival, except for water, which will be allowed up to one hour before their arrival.

Upon arrival, during each of the sessions the children will be given a drink (milk, chocolate milk, drinkable yogurt, sugar-sweetened beverage or water). McCain pizza (purchased at Loblaws) and the same drink as earlier will be served 60 minutes after the boys/girls consume the drink). Children will be told that they may eat as little or as much pizza as they like. The amount of food eaten by each child will be measured.

The boys/girls will also be requested to complete scales on which they will place a pencil mark to describe their desire to eat (“Very weak” to “Very strong”), hunger (“Not hungry at all” to “As hungry as I’ve ever felt”), fullness (“Not full at all” to “Very full”), how much food they could eat (“A large amount” to “Nothing at all”). They will also be asked to complete similar scales on how much they like the drinks and the pizza. They will complete these scales during the information session, in order to become familiar with the test instruments.

Samples of saliva will be non-invasively collected with synthetic swab and tube for analysis of cortisol as an indicator of the child’s stress level

The children will be fully supervised during the study sessions. They will be engaged in age appropriate entertainment (as distraction) e.g.: reading, puzzles, cards, before lunch. The study session will end approximately 60 minutes after the pizza meal.

Confidentiality:

Records relating to participants will be kept confidential in a locked cabinet in the Department of Nutritional Sciences and no disclosure of personal information of the children or parents will take place except where required by law. Participants will have a code and a number that will identify them in all documents, records and files to keep their name confidential. All data will be entered into Microsoft Excel files, available only to investigators. Each participant will have a file, also only available for investigators. All forms and printouts will be stored in the
individual files and clearly labeled. All documents will be kept for a minimum of five years following completion of the study and then securely destroyed.

Risks:

There is very little risk related to this study. The provided breakfast and the served test beverages are commercially available and safe for human consumption. In addition, the pizza that children will be asked to consume are prepared hygienically in the kitchen at the time of the session and present minimal risk. Children may feel dizzy following the overnight fast, but this is rare. If this happens, they will likely feel fine once they consume the breakfast meal provided.

Benefits:

As the causes of obesity remains undefined, the potential benefits from this study will be a better understanding of the regulation food intake in children and might contribute to the prevention of obesity in children.

Questions and further information:

Participation is completely voluntary and failure to participate will not have any consequences. Also, you and your child have the option to stop participating or skip any step/question at any time.

If you have any questions or would like further information concerning this research project, please do not hesitate to call: Ms. Shirley Vien (416) 978-4153, Dr. Bohdan Luhovyy (902) 457-6256 or Dr. Harvey Anderson at (416)-978-1832.

If you have questions or concerns about your rights as a research participant, please contact Dr. Rachel Zand, 416 946 3389, rachel.zand@utoronto.ca.

Dissemination of findings:

A summary of results will be made available to you to pick up after the study is completed.
Consent:

I acknowledge that the research procedures described above and of which I have a copy, have been explained to me and that any questions that I have asked have been answered to my satisfaction. I know that I may ask additional questions now or in the future. I am aware that participation in the study will not involve any health risk to my child.

I understand that for purposes of the research project, if my child or I choose to withdraw from the study at any time, we may do so without prejudice.

Upon completion of each study session, my child will receive a $20 gift certificate to the theatre. The final summary and results of the study will be available for me to pick up from the Department of Nutritional Sciences, University of Toronto. I am aware that the researchers may publish the study results in scientific journals, keeping confidential my son or daughter’s identity.

I hereby consent for my child, ________________________________, to participate in this study.

___________________________________         _____________________________
(Name of parent or guardian)                    (Signature of parent or guardian)

__________________________________           ___________________________
(Name of witness)                                                    (Signature of witness)

Date: _____________ (dd/mm/yy)
Children’s Assent Form

This study will help to find out how good various snacks and drinks are for children’s health. My weight, height, and body fat will be measured without pain during the screening visit. I will be asked to fill out a questionnaire that is related to my stage of puberty (changes in my body as I grow up). I will also be asked to look at some cartoon pictures and pick the one that looks most like me. If I am a girl, I will be asked to answer questions about whether I have started my period. I can ask my parents to answer these questionnaires and pick the picture for me. I will also be asked to drink a drink, complete special scales to show if I am hungry or full. I will also be provided with a pizza lunch during each study session (that I will eat in the Department of Nutritional Sciences, University of Toronto). All the experimental sessions will be on weekends, school holidays or summer break, so I don’t need to be absent from school.

I know that my participation in the study will not involve any health risk to me.

I will be asked to come for the study five times, but if at any time I decide to stop participating, that will be O.K and I have the choice to not answer any question at any time. I understand that the information related to me will be securely stored and not be given to anyone from outside who is not engaged with this study. I know that I will receive a $20 gift certificate to the theatre after completion of each study session, as a “thank you” for my participation.

“I was present when ______________________________ read this form and gave his/her verbal assent.”

______________________________ Signature

Name of the person who obtained assent:
Experiment 2: U of T Parent’s Consent Form

Department of Nutritional Sciences
FitzGerald Building, 150 College Street, 3rd Floor
Toronto, ON M5S 3E2
CANADA

The Effect of Fluid Dairy Products Consumed Before and Within a Mixed Meal on Blood Glucose, Food Intake and Satiety in Normal Weight and Overweight/Obese Children

(Sessions WITH clinical testing)

Study Information Sheet and Parent’s Consent Form

Investigators: Dr. G. Harvey Anderson, Principle Investigator
Department of Nutritional Sciences, University of Toronto
Phone: (416) 978-1832
Email: harvey.anderson@utoronto.ca

Dr. Bohdan Luhovyy, Assistant Professor
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: (902) 457-6256
Email: bohdan.luhovyy@msvu.ca

Dr. Jill Hamilton, Associate Professor
Department of Paediatrics, University of Toronto
Phone: (416) 813-5115
Email: jill.hamilton@sickkids.ca

Dr. Nick Bellissimo, Assistant Professor
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone: (902) 457-6295
Email: nick.bellissimo@msvu.ca

Ms. Barkha Patel, Ph.D. Candidate
Department of Nutritional Science, University of Toronto
Phone: (416) 978-4153
Email: barkha.patel@utoronto.ca
Purpose of Research:

The purpose of this study is to determine the effects of dairy drinks on food intake and blood sugar regulation in 9-14 year-old children. This experiment is being conducted through the Department of Nutritional Sciences at The Hospital for Sick Children by Dr. G. Harvey Anderson (principal investigator). Your son/daughter will be required to attend one screening session to measure physiological parameters and two experimental sessions (to measure blood sugar and insulin) conducted over a 2-week period for a total of 3 visits to The Hospital for Sick Children. Experimental sessions will last a maximum of 3 hours.

The purpose of our research is to develop an understanding of factors affecting the control of food intake and blood glucose in children. Knowing the determinants of the regulation of food intake and blood glucose in children will allow us to develop strategies and recommendations for the prevention of obesity and diabetes.

Procedure:

Screening:

For those parents who express interest in having their son/daughter participate, some information about the child will be requested by telephone. If the child was born at term, is healthy and does not receive any medications, a screening session will be arranged.

During the screening session, the researcher will explain the full details of the study. Parents that give consent to have their son/daughter participate will sign a consent form. The parent will receive copies of the consent form and of the study information sheet. If the child wishes to participate and signs a children’s assent form, his/her weight, height, and body fat using painless techniques, will be measured.
The boys and girls will then be asked to rank their preference for pizza that will be served as the lunch meal at each session.

The children’s physical activity and eating habits will be assessed with Physical Activity Questionnaire and the Dutch Eating Behaviour Questionnaire.

Menstrual Cycle Questionnaire:

Girls will be asked to complete a questionnaire about their menstrual cycle. This information is collected because studies have shown that energy intake and appetite change across the menstrual cycle.

Tanner Staging

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Two methods will be used to estimate the amount of muscle and fat tissue in your child’s body.

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**Bioelectrical Impedance Analysis:** Bioelectrical impedance analysis (BIA), a recently developed technique for measuring body fat content in both adults and children, is simple and painless and is an effective method for measuring body fat in children. BIA is based on measurement of electrical resistance in the body to a tiny current (that the subject cannot feel). The principle of
BIA lies in that muscle mass in the body is a better conductor of electricity than fat, which contains lesser amounts of water and electrolytes.

Appetite, Food Intake and Blood Sugar Assessment:

The boys/girls who participate in this study, will be asked to go to The Hospital for Sick Children, for two individual morning sessions. These sessions will be held on weekends, over two weeks. The children will be brought to the laboratory and returned home by parents only.

On each of the two test days, the children will have a standardized breakfast of cereal, milk and juice at home, either at 8:00 am or 9:00 am (the time will be consistent for each child). The children will arrive at the FitzGerald Building, either at 10:00 am or 11:00 am (but consistent throughout for each child).

Children will fast for 12 hours before breakfast and after breakfast until their arrival, except for water, which will be allowed up to one hour before their arrival.

Upon arrival, during each of the sessions the children will be given a drink (milk or sugar-sweetened beverage). McCain pizza (purchased at Loblaws) and the same drink as earlier will be served 60 minutes after the boys/girls consume the drink). Children will be told that they may eat as little or as much pizza as they like. The amount of food eaten by each child will be measured.

The boys/girls will also be requested to complete scales on which they will place a pencil mark to describe their desire to eat (“Very weak” to “Very strong”), hunger (“Not hungry at all” to “As hungry as I’ve ever felt”), fullness (“Not full at all” to “Very full”), how much food they could eat (“A large amount” to “Nothing at all”). They will also be asked to complete similar scales on how much they like the drinks and the pizza. They will complete these scales during the information session, in order to become familiar with the test instruments.

At each study session, blood will sampled and used to measure blood sugar and appetite-controlling (hunger) hormones. Ten blood samples (3.5 ml) will be taken during each experimental session. The total volume of blood collected at one session will be 35 ml and the total volume of blood collected within two weeks will be 70 ml. To obtain blood samples, a nurse will insert a catheter (a needle attached to a plastic tube) into a vein in your child’s arm. The catheter will remain in his/her arm and be used to sample blood in small amounts during the session. After the nurse collects the first sample at baseline (0 minutes), your child will consume one of the drinks within five minutes. After they finish the drink, we will collect blood samples at 10, 20, 30, 60, 85, 95, 105, 115 and 145 minutes after baseline.

Samples of saliva will be non-invasively collected with synthetic swab and tube for analysis of cortisol as an indicator of the child’s stress level

The children will be fully supervised during the study sessions. They will be engaged in age appropriate entertainment (as distraction) e.g.: reading, puzzles, cards, before lunch. The study session will end approximately 60 minutes after the pizza meal.
Confidentiality:

Records relating to participants will be kept confidential in a locked cabinet in the Department of Nutritional Sciences and no disclosure of personal information of the children or parents will take place except where required by law. Participants will have a code and a number that will identify them in all documents, records and files to keep their name confidential. All data will be entered into Microsoft Excel files, available only to investigators. Each participant will have a file, also only available for investigators. All forms and printouts will be stored in the individual files and clearly labeled. All documents will be kept for a minimum of five years following completion of the study and then securely destroyed.

Risks:

There is very little risk related to this study. The provided breakfast and the served test beverages are commercially available and safe for human consumption. In addition, the pizza that children will be also asked to consume are prepared hygienically in the kitchen at the time of the session and present minimal risk. Children may feel dizzy following the overnight fast, but this is rare. If this happens, they will likely feel fine once they consume the breakfast meal provided. There is the possibility of a small amount of bruising, pain and the possibility of infection associated with blood collection.

Benefits:

As the causes of obesity remains undefined, the potential benefits from this study will be a better understanding of the regulation food intake in children and might contribute to the prevention of obesity in children.

Questions and further information:

Participation is completely voluntary and failure to participate will not have any consequences. Also, you and your child have the option to stop participating or skip any step/question at any time.

If you have any questions or would like further information concerning this research project, please do not hesitate to call: Ms. Shirley Vien (416) 978-4153, Dr. Bohdan Luhovyy (902) 457-6256 or Dr. Harvey Anderson at (416)-978-1832.

If you have questions or concerns about your rights as a research participant, please contact Dr. Rachel Zand, 416 946 3389, rachel.zand@utoronto.ca.

Dissemination of findings:

A summary of results will be made available to you to pick up after the study is completed.
Consent:

I acknowledge that the research procedures described above and of which I have a copy, have been explained to me and that any questions that I have asked have been answered to my satisfaction. I know that I may ask additional questions now or in the future. I am aware that participation in the study will not involve any health risk to my child.

I understand that for purposes of the research project, if my child or I choose to withdraw from the study at any time, we may do so without prejudice.

Upon completion of each study session, my child will receive a $50 gift certificate to the theatre or bookstore. The final summary and results of the study will be available for me to pick up from the Department of Nutritional Sciences, University of Toronto. I am aware that the researchers may publish the study results in scientific journals, keeping confidential my son or daughter’s identity.

I hereby consent for my child, _____________________________________, to participate in this study.

___________________________________         _____________________________
(Name of parent or guardian)                                  (Signature of parent or guardian)

__________________________________           ___________________________
(Name of witness)                                  (Signature of witness)

Date: ______________ (dd/mm/yy)
7.5.4 Experiment 2: U of T Child’s Assent Form

Department of Nutritional Sciences
FitzGerald Building, 150 College Street, 3rd Floor
Toronto, ON M5S 3E2
CANADA

The Effect of Fluid Dairy Products Consumed Before and Within a Mixed Meal on Blood Glucose, Food Intake and Satiety in Normal Weight and Overweight/Obese Children

(Sessions WITH clinical testing)

Children’s Assent Form

This study will help to find out how good various snacks and drinks are for children’s health. My weight, height, and body fat will be measured without pain during the screening visit. I will be asked to fill out a questionnaire that is related to my stage of puberty (changes in my body as I grow up). I will also be asked to look at some cartoon pictures and pick the one that looks most like me. If I am a girl, I will be asked to answer questions about whether I have started my period. I can ask my parents to answer these questionnaires and pick the picture for me. I will also be asked to drink a drink, complete special scales to show if I am hungry or full and have blood and saliva samples taken. I will also be provided with a pizza lunch during each study session (that I will eat in The Hospital for Sick Children). All the experimental sessions will be on weekends, school holidays or summer break, so I don’t need to be absent from school.

I know that my participation in the study will not involve any health risk to me.

I will be asked to come for the study two times, but if at any time I decide to stop participating, that will be O.K and I have the choice to not answer any question at any time. I understand that the information related to me will be securely stored and not be given to anyone from outside who is not engaged with this study. I know that I will receive a $50 gift certificate to the theatre or bookstore after completion of each study session, as a “thank you” for my participation.

“I was present when ____________________________ read this form and gave his/her verbal assent.”

____________________________ Signature

Name of the person who obtained assent:
7.5.5 Experiment 2: Sick Kids Parent Consent Form

Parent/Caregiver Consent Form

Title of Research Project:

The Effect of Fluid Dairy Products Consumed Before and Within a Mixed Meal on Blood Glucose, Food Intake and Satiety in Normal Weight and Overweight/Obese Children

Investigator(s):

Dr. Jill Hamilton, Principal Investigator
Staff Physician, Division of Endocrinology, The Hospital for Sick Children
Senior Associate Scientist, SickKids Research Institute Department of Paediatrics, University of Toronto
Phone: (416) 813-5115
Email: jill.hamilton@sickkids.ca

Dr. G. Harvey Anderson, Co-Investigator
Department of Nutritional Sciences, University of Toronto
Phone: (416) 978-1832
Email: harvey.anderson@utoronto.ca

Study Coordinators:

Munaza Jamil (416) 813-7654 x28363
Rachel Steger (416) 813-7654 x28363
Shirley Vien (416) 978-4153

Purpose of the Research:

The purpose of our research is to better understand factors affecting the control of food intake and blood glucose in children. If we can understand more about how the kinds of food we eat affects our regulation of food intake and blood glucose, it will allow us to develop strategies and recommendations for the prevention of obesity and diabetes.

The purpose of this study is to find out what effects dairy drinks/snacks have on food intake and blood sugar regulation in normal and overweight 9-14 year-old children. We will do this research at two sites in Toronto; the Hospital for Sick Children and the Department of Nutritional Sciences at the University of Toronto.
**Description of the Research:**

Subjects will be screened during the information session at University of Toronto. During the information session, we will explain the full details of the study. If you would like your child to participate, you will sign a consent form and your child will give assent. You will receive a copy of the consent form and a study information sheet. Once consent is obtained, your child’s weight, height and body fat will be measured. In addition your child will be asked to complete a number of questionnaires related to their pubertal stage and menstrual cycle (girls only), as well as their activity levels and eating habits.

In order to determine if snacking on milk products will affect blood sugar regulation, hunger and energy intake, we must compare a group of people who consume milk with a group of people who do not consume milk products. Your child will be randomly assigned to receiving milk on one visit and juice on the second visit. Both your child and their health care team will know which drink your child received. The method we use to decide which drink you get is called randomization. This means that no one makes the choice; it is done randomly, by chance, like tossing a coin.

If you have agreed to the blood work portion of the study, the testing portion of this study will consist of 2 visits over a 2 week period at the Hospital for Sick Children. The SickKids study will be carried out in the Physiological Research Unit (PRU) and will be performed on 2 single day visits, with participants arriving at 9:00am and finishing by 1:00pm. Each morning visit will last 4 hours, the total time required to participate in both visits is 8 hours.

At the information session you will be given a standardized breakfast to give your child the morning of his or her visit to SickKids. Your child will fast for 12 hours before breakfast and may only have water after breakfast until they arrive at SickKids at 9:00am.

When you arrive a study team member will offer your child some cream (EMLA) to numb the skin where the needle will be inserted. A nurse will then insert a catheter (a needle attached to a plastic tube) into a vein in your child’s arm. The catheter will remain in their arm and will be used to collect blood samples in order to measure your child’s blood sugar and insulin levels. In total, blood will be collected at 10 time points, and only 3.5 mL of blood (less than one teaspoon) will be collected at each time point. After the nurse collects the first sample at baseline (0 minutes), your child will be given a snack (milk or juice) and will be ask to drink it within five minutes. The nurse will collect blood samples and your child will complete a questionnaire to assess how hungry they are at 10, 20, 30, and 60 minutes after baseline. Your child will then be provided with a pizza lunch and the same drink they had earlier; the amount of pizza they eat will be measured. We will also continue taking blood samples and doing questionnaires at 85, 95, 105, 115 and 145 minutes after baseline. All of the blood samples will be taken through the intravenous line so there will be only one poke with the needle.

Below is an outline of the visits to help you better understand the flow of the testing day and give you information about where to go and how long each test will take.
<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00 am</td>
<td>Arrival at Sick Kids (PRU)</td>
</tr>
<tr>
<td>10:00 am</td>
<td>Blood sample collection, questionnaire completion and drink provided</td>
</tr>
<tr>
<td>11:00 am</td>
<td>Pizza lunch, blood sample collection and questionnaire completion</td>
</tr>
<tr>
<td>1:00 pm</td>
<td>Visit over</td>
</tr>
</tbody>
</table>

**Potential Harms:**
There is very little known harm related to this study. There may be a small amount of bleeding when blood is taken from a vein and there may be slight discomfort, bruising or redness that will usually disappear in a few days. We will offer a special cream (EMLA) that can be applied to the skin to numb it and reduce the discomfort prior to the needle poke.

**Potential Discomforts or Inconvenience:**
Potential inconveniences will include travelling to SickKids on a morning for 4 hours each week for two weeks.

**Potential Benefits:**

**To individual subjects:**
Your child will not benefit directly from participating in this study. A summary of results will be made available to you to pick up after the study is completed.

**To society:**
The potential benefits from this study are a better understanding of food intake and glycemic (glucose) control in children, which is very important for the prevention and treatment of obesity and related chronic diseases.

**Alternatives to participation:**
Participation is completely voluntary and choosing not to participate will not have any consequences. You and your child have the option to stop participating or skip any step/question at any time. If you or your child does not want to have blood samples taken you may still participate in the University of Toronto portion of the study which will include 5 treatment visits.

If you have any questions or would like further information concerning this research project, please do not hesitate to call Dr. Jill Hamilton (416) 813-5115 or Dr. Harvey Anderson at (416)-978-1832.

**Confidentiality:**
We will respect your privacy. No information about who your child is will be given to anyone or be published without your permission, unless the law requires us to do this. For example, the law requires us to give information about your child if a child has been abused, if your child
has an illness that could spread to others, if you or someone else talks about suicide (killing themselves), or if the court orders us to give them the study papers

SickKids Clinical Research Monitors, employees of the funder or sponsor of the study Dairy Farmers of Canada, or the regulator of the study may see your child’s health or research record to check on the study. For example, people from Health Canada Health Products and Food Branch, if necessary, may look at your records.

By signing this consent form, you agree to let these people look at your child’s health or research records. We will put a copy of this research consent form in your child’s research record. We will give you a copy for your files.

The data produced from this study will be stored in a secure, locked location. Only members of the research team (and maybe those individuals described above) will have access to the data and your child’s blood samples. This could include external research team members. Following completion of the research study, the data will be kept as long as required and then destroyed as required by Sick Kids policy. Your child’s blood samples will de-identified and shipped to Mount Sinai for insulin and glucose analysis. Published study results will not reveal your child’s identity.

**Reimbursement:**

Parents will be compensated $12 per visit for a session for travel costs (TTC/parking). If your child stops taking part in the study, we will pay you for your expenses for taking part in the study up until that point.

As a reward for your child’s participation, they will receive a certificate of participation and a $50 gift certificate to the movie theatre or book store after each study session at SickKids in recognition of their time and effort.

**Participation:**

If your child does not feel comfortable with having blood taken, they can withdraw from the study and take part in the University of Toronto portion that does not require blood and will involve 5 treatment visits. Or, you can take your child out of the study at any time. The care your child gets at Sick Kids will not be affected in any way by whether your child takes part in this study.

New information that we get while we are doing this study may affect your decision to take part in this study. If this happens, we will tell you about this new information. And we will ask you again if you still want to be in the study.

Your child will be required to read and sign an assent form and make it known they understand the study they will participate in.

In some situations, the study doctor or the company paying for the study may decide to stop the study. This could happen even if the treatment given in the study is helping your child. If this happens, the study doctor will talk to you about what will happen next.
If your child becomes ill or are harmed because of study participation, we will treat your child for free. Your signing this consent form does not interfere with your legal rights in any way. The study staff, any people who gave money for the study, or the hospital are still responsible, legally and professionally, for what they do.

**Sponsorship:**

This study is funded and sponsored by the Dairy Farmers of Canada and Dr Jill Hamilton of The Hospital for Sick Children.

**Conflict of Interest:**

Dr Jill Hamilton, and the other research team members, have no conflict of interest to declare

**Future Contact:**

Do you give permission on behalf of your child to be contacted for future follow up studies that are conducted?

Yes, I do [ ] No, I do not [ ]

**Information to be used for Future Analyses:**

Do you give permission on behalf of your child for his or her information to be used anonymously in future studies for subsequent analyses?

Yes, I do [ ] No, I do not [ ]

Do you give permission to use any blood samples collected in this study for future appetite hormone analyses.

Yes, I do [ ] No, I do not [ ]

**Educational Training Purposes:**

Do you give permission on behalf of your child for his or her information to be used anonymously in future studies for educational training purposes?

Yes, I do [ ] No, I do not [ ]
Consent:

By signing this form, I agree that:
1) You have explained this study to me. You have answered all my questions.
2) You have explained the possible harms and benefits (if any) of this study.
3) I know what I could do instead of having my child take part in this study. I understand that I have the right to refuse to let my child take part in the study. I also have the right to take my child out of the study at any time. My decision about my child taking part in the study will not affect my child’s health care at Sick Kids.
4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my child’s medical records will be kept private except as described to me.
6) I understand that no information about my child will be given to anyone or be published without first asking my permission.
7) I have read pages 1 to 6 and I agree, or consent, that my child_______________________ may take part in this study.

________________________________________________________________________
Printed Name of Parent/Legal Guardian

________________________________________________________________________
Parent/Legal Guardian’s signature & date

________________________________________________________________________
Printed Name of person who explained consent

________________________________________________________________________
Signature of Person who explained consent & date

________________________________________________________________________
Printed Witness’ name (if the subject/legal guardian does not read English)

________________________________________________________________________
Witness’ signature & date

If you have any questions about this study, please call Ms. Shirley Vien at 416-978-4153

If you have questions about your rights as a subject in a study or injuries during a study, please call the Research Ethics Manager at 416-813-5718.
7.5.6 Experiment 2: Sick Kids Participant Consent Form

**Participant Consent Form**

**Title of Research Project:**

The Effect of Fluid Dairy Products Consumed Before and Within a Mixed Meal on Blood Glucose, Food Intake and Satiety in Normal Weight and Overweight/Obese Children

**Investigator(s):**

Dr. Jill Hamilton, Principal Investigator  
Staff Physician, Division of Endocrinology, The Hospital for Sick Children  
Senior Associate Scientist, SickKids Research Institute  
Department of Paediatrics, University of Toronto  
Phone: (416) 813-5115  
Email: jill.hamilton@sickkids.ca

Dr. G. Harvey Anderson, Co-Investigator  
Department of Nutritional Sciences, University of Toronto  
Phone: (416) 978-1832  
Email: harvery.anderson@utoronto.ca

**Study Coordinators:**

Munaza Jamil (416) 813-7654 x28363  
Rachel Steger (416) 813-7654 x28363  
Shirley Vien (416) 978-4153

**Purpose of the Research:**

The purpose of our research is to better understand factors affecting the control of food intake and blood glucose in children. If we can understand more about how the kinds of food we eat affects our regulation of food intake and blood glucose in children, it will allow us to develop strategies and recommendations for the prevention of obesity and diabetes.

The purpose of this study is to find out what effects dairy drinks have on food intake and blood sugar regulation in normal weight as well as overweight and obese 9-14 year-old children. We will do this research at two sites in Toronto; the Hospital for Sick Children and the Department of Nutritional Sciences at the University of Toronto.
Description of the Research:

Subjects will be screened during the information session at University of Toronto. During the information session, we will explain the full details of the study. If you would like to participate in the SickKids portion, you will sign a consent form. You will receive a copy of the consent form and a study information sheet. Once consent is obtained, your weight, height and body fat will be measured. In addition you will be asked to complete a number of questionnaires related to your pubertal stage and menstrual cycle (girls only), as well as your activity levels and eating habits.

In order to determine if snacking on milk products will affect blood sugar regulation, hunger and energy intake, we must compare a group of people who consume milk with a group of people who do not consume milk products. You will be randomly assigned to receiving milk on one visit and juice on the second visit. Both you and your health care team will know which drink you received. The method we use to decide which drink you get is called randomization. This means that no one makes the choice; it is done randomly, by chance, like tossing a coin.

If you have agreed to the bloodwork portion of the study, the testing portion of this study will consist of 2 visits over a 2 week period at the Hospital for Sick Children. The SickKids study will be carried out in the Physiological Research Unit (PRU) and will be performed on 2 single morning visits, with participants arriving at 9:00am and finishing by 1:00pm. Each morning visit will last 4 hours, the total time required to participate in both visits is 8 hours.

At the information session you or your parents will be given a standardized breakfast for you to eat the morning of your visit to SickKids. You will fast for 12 hours before breakfast and may only have water after breakfast until you arrive at SickKids at 9:00am.

When you arrive a study team member will offer you some cream (EMLA) to numb the skin where the needle will be inserted. A nurse will then insert a catheter (a needle attached to a plastic tube) into a vein in your arm. The catheter will remain in your arm and will be used to collect blood samples in order to measure your blood sugar and insulin levels. In total, blood will be collected at 10 time points, and only 3.5 mL of blood (less than one teaspoon) will be collected at each time point. After the nurse collects the first sample at baseline (0 minutes), you will be given a snack (milk or juice) and will be asked to drink it within five minutes. The nurse will collect blood samples and you will complete a questionnaire to assess how hungry you are at 10, 20, 30, and 60 minutes after baseline. You will then be provided with a pizza lunch and the same drink you had earlier; the amount of pizza you eat will be measured, eat as much as you like. You will also complete some questionnaires about your comfort level and hunger. We will continue taking blood samples and doing questionnaires at 85, 95, 105, 115 and 145 minutes after baseline. All of the blood samples will be taken through the intravenous line so there will be only one poke with the needle.

Below is an outline of the visits to help you better understand the flow of the testing day and give you information about where to go and how long each test will take.
9:00 am | Arrival at Sick Kids (PRU)
10:00 am | Blood sample collection, questionnaire completion and drink provided
11:00 am | Pizza lunch, blood sample collection and questionnaire completion
1:00 pm | Visit over

**Potential Harms:**

There is very little known harm related to this study. There may be a small amount of bleeding when blood is taken from a vein and there may be slight discomfort, bruising or redness that will usually disappear in a few days. We will offer a special cream (EMLA) that can be applied to the skin to numb it and reduce the discomfort prior to the needle poke.

**Potential Discomforts or Inconvenience:**

Potential inconveniences will include travelling to SickKids on a weekend morning for 4 hours each week for two weeks.

**Potential Benefits:**

**To individual subjects:**

You will not benefit directly from participating in this study. A summary of results will be made available to you to pick up after the study is completed.

**To society:**

The potential benefits from this study are a better understanding of food intake and glycemic (glucose) control in children, which is very important for the prevention and treatment of obesity and related chronic diseases.

**Alternatives to participation:**

Participation is completely voluntary and choosing not to participate will not have any consequences. You have the option to stop participating or skip any step/question at any time. If you do not want to have blood samples taken you may still participate in the University of Toronto portion of the study which will include 5 treatment visits.

If you have any questions or would like further information concerning this research project, please do not hesitate to call Dr. Jill Hamilton (416) 813-5115 or Dr. Harvey Anderson at (416)-978-1832.
Confidentiality:

We will respect your privacy. No personal information will be given to anyone or be published without your permission, unless the law requires us to do this. For example, the law requires us to report information if you have been abused, if you have an illness that could spread to others, if you or someone else talks about suicide (killing themselves), or if the court orders us to give them the study papers.

SickKids Clinical Research Monitors, employees of the funder or sponsor of the study Dairy Farmers of Canada, or the regulator of the study may see your health or research record to check on the study. For example, people from Health Canada Health Products and Food Branch, if necessary, may look at your health or research record.

By signing this consent form, you agree to let these people look at your records. We will put a copy of this research consent form in your research record. We will give you a copy for your files.

The data produced from this study will be stored in a secure, locked location. Only members of the research team (and maybe those individuals described above) will have access to the data. This could include external research team members. Following completion of the research study, the data will be kept as long as required and then destroyed as required by Sick Kids.

Your blood samples will be identified and shipped to Mount Sinai for insulin and glucose analysis. Published study results will not reveal your identity.

Reimbursement:

You or your parents will be compensated $12 per visit for a session for travel costs (TTC/parking).

As a reward for your participation, you will receive a certificate of participation and a $50 gift certificate to the movie theatre or book store after each study session at SickKids in recognition of your time and effort.

Participation:

If you do not feel comfortable with having blood taken, you can withdraw from the study and take part in the University of Toronto portion that does not require blood and will involve 5 treatment visits.

Or, if you choose to take part in this study you can withdraw from the study at any time. The care you get at Sick Kids will not be affected in any way by whether you take part in this study.

New information that we get while we are doing this study may affect your decision to take part in this study. If this happens, we will tell you about this new information. And we will ask you again if you still want to be in the study.
In some situations, the study doctor or the company paying for the study may decide to stop the study. This could happen even if the treatment given in the study is helping you. If this happens, the study doctor will talk to you about what will happen next.

If you become ill or are harmed because of study participation, we will treat you for free. Your signing this consent form does not interfere with your legal rights in any way. The study staff, any people who gave money for the study, or the hospital are still responsible, legally and professionally, for what they do.

**Sponsorship:**

This study is funded and sponsored by the Dairy Farmers of Canada and by Dr Jill Hamilton of The Hospital for Sick Children.

**Conflict of Interest:**

Dr Jill Hamilton, and the other research team members, have no conflict of interest to declare.

**Future Contact:**

Do you give permission to be contacted for future follow up studies that are conducted?

Yes, I do ☐  No, I do not ☐

**Information to be used for Future Analyses:**

Do you give permission your information to be used anonymously in future studies for subsequent analyses?

Yes, I do ☐  No, I do not ☐

Do you give permission to use any blood samples collected in this study for future appetite hormone analyses.

Yes, I do ☐  No, I do not ☐

**Educational Training Purposes:**

Do you give permission for your information to be used anonymously in future studies for educational training purposes?

Yes, I do ☐  No, I do not ☐
Consent:

By signing this form, I agree that:
1) You have explained this study to me. You have answered all my questions.
2) You have explained the possible harms and benefits (if any) of this study.
3) I know what I could do instead of taking part in this study. I understand that I have the right not to take part in the study and the right to stop at any time. My decision about taking part in the study will not affect my health care at Sick Kids.
4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my medical records will be kept private except as described to me.
6) I understand that no information about who I am will be given to anyone or be published without first asking my permission.
7) I have read pages 1 to 6 and I agree, or consent, to take part in this study.

___________________________
Printed Name of Subject & Age
Subject’s signature & date

___________________________
Printed Name of person who explained consent
Signature of Person who explained consent & date

Printed Witness’ name (if the subject/legal guardian does not English)
Witness’ signature & date

If you have any questions about this study, please call Ms. Shirley Vien at 416-978-4153

If you have questions about your rights as a subject in a study or for information on whom to contact in the event of injuries during a study, please call the Research Ethics Manager at 416-813-5718.
7.5.7 Experiment 2: Sick Kids Assent Form

**Title of Research Project:**
The Effect of Fluid Dairy Products Consumed Before and Within a Mixed Meal on Blood Glucose, Food Intake and Satiety in Normal Weight and Overweight/Obese Children

**Principal Investigator:**
Dr. Jill Hamilton  
Phone: 416.813.5115  
Email: jill.hamilton@sickkids.ca

**Co-Investigator:**
Dr. G. Harvey Anderson  
Phone: (416) 978-1832  
Email: harvery.anderson@utoronto.ca

**Study Coordinators:**
Shirley Vien  
Phone: (416) 978-4153  
Email: shirley.vien@utoronto.ca

Munaza Jamil  
Phone: (416) 813-7654 x28363

Rachel Steger  
Phone: (416) 813-7654 x28363

**Why are we doing this study?**
The number of overweight and obese children has increased significantly in the last 30 years. We want to know if drinking milk instead of juice will help children get and maintain a healthy weight by making them less hungry at meal times. This may help kids keep a healthy weight.

**What will happen during the study?**
1) Your parents will give you a special breakfast at home. You will not be able to eat for 12 hours before this breakfast
2) You will come to Sick Kids later that morning and meet a nurse. She will give you a special blood test to test the amount of sugar in your blood.
3) You will be given a snack (milk or juice) and you will be asked to drink it...
4) We will do some more blood tests and you will be asked some questions about how hungry you are
5) You will get a pizza lunch
6) You will be asked to answer questions about changes to your body related to puberty

Are there good things and bad things about the study?
You will help doctors learn more about how much children eat. This may help prevent and treat overweight children.

When you come to Sick Kids for the testing you may also feel some pain or have a small bruise when we take your blood with a needle. You can use special cream to numb the skin so the poke does not hurt as much.

Who will know about what I did in the study?
If we feel your health may be in danger, we may have to give your results to your doctor.

Can I decide if I want to be in the study?
Yes, you decide whether or not you want to participate. Nobody will be angry or upset if you do not want to be in the study. If you would like to participate in the study but do not want to do the medical testing at Sick Kids, that is your choice. We are talking to your parent/legal guardians about the study and you should talk to them about it too.

**Assent:**
The following section must be included at the end of the assent form:

I was present when ___________________________ read this form and said that he or she agreed, or assented, to take part in this study.

_____________________________  _________________________
Printed Name of person who obtained assent  Signature & Date
Appendix 6. Screening Questionnaires

7.6.1 Telephone Screening Questionnaire

UNIVERSITY OF TORONTO
DEPARTMENT OF NUTRITIONAL SCIENCES

Pre-meal Snacks, Satiety and Food Intake in Children.
Part A or Part B (circle correct one)

Name: ________________________________
Age: ________ years DOB (d/m/y) _________ Term baby? Yes / No
Height: ________ cm Weight: ____________ kg Normal birth weight? Yes / No
Has your child gained or lost weight recently? Yes / No (circle correct answer)
Does your child usually have breakfast? Yes / No
Does your child like (foods that will be used in study)
milk Yes / No cereal Yes / No grapes Yes / No almonds Yes / No
juice Yes / No pizza Yes / No raisins Yes / No yogurt Yes / No
Is your child following a special diet? Yes / No
Does your child have food allergies or sensitivities? Yes / No
Health problems? Yes / No
If yes, which problem? ________________________________
Medication/s? Yes / No
If yes, which medication/s? ________________________________
Education: Grade: ________ Special class? Yes / No
Skipped or repeated grade? Yes / No Learning difficulties/problems? Yes / No
Behavioral or emotional problems Yes / No
If yes, which problem? ________________________________
Include in study? Yes / No
If not, why? ________________________________
Appointment date: ________________ (d/m/y)
Investigator: ______________________ Date: __________ (d/m/y)
7.6.2 Background Information Questionnaire

Department of Nutritional Sciences, University of Toronto
Pre-meal Snacks, Satiety and Food Intake in Children

Background Information

Child’s Name: ____________________________________________

Child’s Date of Birth: ________________________________ (dd/mm/yy)

Child’s Sex (Circle One): MALE / FEMALE

If FEMALE, has your child begun to menstruate? YES / NO

What age was your child when she had her first period? ________ Years-old

Ethnic Background: ________________________________

Mother’s Weight: ________ Kg / lb

Height: _________ cm / inches

Father’s Weight: ________ Kg / lb

Height: _________ cm / inches

(Circle correct unit)

Contact Information

Address: ____________________________________________

____________________________________________________

____________________________________________________

Home Phone #: ________________________________

Mother’s Name: ____________________________________________

Cell Phone #: ________________________________

Work Phone #: ________________________________

Father’s Name: ____________________________________________

Cell Phone #: ________________________________

Work Phone #: ________________________________

Source of referral: ____________________________________________

Subject ID: ____________________________________________
7.6.3  Food Acceptability List Questionnaire

Investigator: ___________________________ Date: ___________ (d/m/y)

Food Acceptability List
Department of Nutritional Sciences, University of Toronto

Pre-meal Snacks, Satiety and Food Intake in Children

Name: ___________________________ Birth Date: __________________

BREAKFAST
On each test day you will eat the same breakfast at home given to you by the investigators.
Please indicate whether you will be able to eat the following:
1 cup nonfat milk (250 mL) Yes / No
Honey Nut Cheerios (26 g) Yes / No  (Circle one Yes OR No)
Junior Tropicana orange juice (236 mL) Yes / No

BEVERAGE
Please indicate whether you will be able to drink the beverages below:
2% Milk (250 mL) Yes / No
Chocolate Milk (191 mL) Yes / No  (Circle one Yes OR No)
Drinkable Yogurt (173 mL) Yes / No
Juice (232 mL) Yes / No

LUNCH
You will be given a pizza lunch on the day of the study.
For us to provide you with a lunch you will enjoy, please circle what you would like to eat (circle a, b OR c):
(a) All PEPPERONI pizza (cheese & pepperoni
(b) All CHEESE pizza (3-cheese: mozzarella, cheddar and parmesean)
(c) A COMBINATION of pepperoni & 3-cheese pizza

If you answered (c), please circle what you would like more:
pepperoni OR 3-cheese  
(Circle One)

Subject ID: ___________________________

Investigator: ___________________________ Date: ___________ (d/m/y)
7.6.4 Puberty Questionnaire

Puberty Questionnaire (Self-administered)

Would you say that your growth spurt (height):
1. there has been no development
2. development has barely begun
3. development is definitely underway
4. development is already completed

And regarding hair growth (under your arms, your pubic hair), would you say that:
1. there has been no development
2. development has barely begun
3. development is definitely underway
4. development is already completed

Have you noticed changes in your skin (e.g. acne)?
1. there have been no changes
2. changes have barely begun
3. changes are definitely underway
4. changes are already complete

FOR GIRLS: FOR BOYS:

Have your breasts started to develop?
1. there has been no development
2. development has barely begun
3. development is definitely underway
4. development is already completed

Have you noticed that your voice has changed (lowered)?
1. there have been no changes
2. changes have barely begun
3. changes are definitely underway
4. changes are already complete

Have you started to have hair on your face?
1. there have been no changes
2. changes have barely begun
3. changes are definitely underway
4. changes are already complete

*NOTE: Girls with menarche start within a year of study visit = Tanner 4, girls with menarche start over one year of study visit = Tanner 5.

Tanner stage exam be MD for boys and premenarchial girls.
If subject refuses exam, self-stage with cartoons and tanner beads (for boys).
7.6.5 Tanner Staging: Male

Tanner Staging
7.6.6 Tanner Staging: Female

Tanner Staging
Tanner Staging
7.6.7 Menstrual Cycle Questionnaire

Menstrual Cycle Questionnaire

1. When were you born? ________________________________

2. Have you had your first period? ____________________

If you answered no, you are finished this questionnaire.

If you answered yes, please complete the following questions.

3. How old were you when you had your first period?
   I was _____ years old when I had my first period.

4. Do you remember the day or month of your first period? ________________

5. How long is your average menstrual cycle? (from the beginning of menstrual flow [menses] to the beginning of the next menstrual flow [menses])
   My average cycle length is ____ days.

6. Currently, for how many days do you typically experience menstrual flow each cycle?
   ____1 day  ____2 days  ____3 days  ____4 days  ____5 days  ____> 5+ days

7. In the past 3 months, estimate how many menstrual cycles you have had?
   I have had _____ cycles in the past 3 months

8. In the past 6 months, estimate how many menstrual cycles you have had?
   I have had _____ cycles in the past 6 months

9. In the past 9 months, estimate how many menstrual cycles you have had?
   I have had _____ cycles in the past 9 months

10. In the past 12 months, estimate how many menstrual cycles you have had?
    I have had _____ cycles in the past 12 months

11. How would you characterize your menstrual flow in the first two days of menses?
    Circle one: Heavy  Moderate  Light

12. Do you experience cramps during menses?
    Circle One: Always  Sometimes  Never
13. Do you typically experience any pain during the middle of your cycle?

Circle one: Always          Sometimes          Never

14. Do you typically experience spotting or sporadic bleeding not associated with normal menstrual flow?

Circle one: Always          Sometimes          Never
7.6.8 Dutch Eating Habits Questionnaire

1. Subject and test details

Name: ____________________________________________________________

Date of birth: ______________________________________________________

Age: _____________________________________________________________

Gender: □ male □ female

Today’s date: ______________________________________________________

2. Your weight, height, etc.

A. Current weight (kg): __________________________

B. Current height (cm): __________________________

C. Has your body weight been constant over the past six months?
   □ yes, my weight did not change much
   □ no, I lost ________ kg
   □ no, I gained ________ kg
   □ no, sometimes I gained weight and sometimes I lost weight

D. Have you ever had an episode of eating an amount of food that others would regard as unusually large?
   □ yes
   □ no

Please do not mark below this line

BMI (please take the age of the child into account): __________________________

<table>
<thead>
<tr>
<th>DEBQ scale</th>
<th>Raw score</th>
<th>Number of items</th>
<th>Scale score</th>
<th>Classification</th>
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<tbody>
<tr>
<td>Emotional eating</td>
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<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External eating</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restrained eating</td>
<td></td>
<td>7</td>
<td></td>
<td></td>
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</tbody>
</table>

Please turn over >>>>>>
**Instructions**
Below you’ll find 20 questions about eating.
Please read each question carefully and tick the answer that suits you best.
Only one answer is allowed. Don’t skip any answer.
There are no incorrect answers; it’s your opinion that counts.

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>No</th>
<th>Sometimes</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Do you feel like eating whenever you see or smell good food?</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2.</td>
<td>If you feel depressed do you get a desire for food?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3.</td>
<td>If you feel lonely do you get a desire for food?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4.</td>
<td>Do you keep an eye on exactly what you eat?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5.</td>
<td>Does walking past a candy store make you feel like eating?</td>
<td></td>
<td></td>
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<tr>
<td>6.</td>
<td>Do you intentionally eat food that helps you lose weight?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Does watching others eat make you feel like eating too?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>If you have eaten too much do you eat less than usual the next day?</td>
<td></td>
<td></td>
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<tr>
<td>9.</td>
<td>Does worrying make you feel like eating?</td>
<td></td>
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<tr>
<td>10.</td>
<td>Do you find it difficult to stay away from delicious food?</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>11.</td>
<td>Do you intentionally eat less to avoid gaining weight?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>If things go wrong do you get a desire for food?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Do you feel like eating when you walk past a restaurant or fast food restaurant?</td>
<td></td>
<td></td>
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<tr>
<td>14.</td>
<td>Have you ever tried not to eat in between meals to lose weight?</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>15.</td>
<td>Do you have a desire to eat when you feel restless?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>16.</td>
<td>Have you ever tried to avoid eating after your evening meal to lose weight?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>17.</td>
<td>Do you have a desire for food when you are afraid?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>Do you ever think that food will be fattening or slimming when you eat?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>19.</td>
<td>If you feel sorry do you feel like eating?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>20.</td>
<td>If somebody prepares food do you get an appetite?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PLEASE CHECK, TO BE SURE THAT YOU TICKED EVERY QUESTION.**
PAST YEAR PHYSICAL ACTIVITY

Check all the activities that you did at least ten times in the PAST YEAR. Include times spent in school physical education classes. Make sure you include all sport teams that you participated in during the last year.

- Aerobics
- Band/Drill Team
- Baseball
- Basketball
- Bicycling
- Bowling
- Cheerleading
- Dance Class
- Football
- Garden/Yard Work
- Gymnastics
- Hiking
- Ice Skating
- Roller Skating
- Running for Exercise
- Skateboarding
- Snow Skiing
- Soccer
- Softball
- Street Hockey
- Swimming (Laps)
- Tennis
- Volleyball
- Water Skiing
- Weight Training
- Wrestling (Competitive)
- Others

List each activity that you checked above in the “Activity” box below, check the months you did each activity and then estimate the amount of time spent in each activity.

| Activity   | J  | F  | M  | A  | P  | A  | N  | B  | R  | Y  | N  | L  | G  | O  | T  | V  | E  | C  | D  | E  | C  | Months Per Year | Days Per Week | Minutes Per Day |
|------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|                |               |                |
|            |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |                |               |                |
|            |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |                |               |                |
|            |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |                |               |                |
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|            |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |                |               |                |
# Body Measurements

**Department of Nutritional Sciences, University of Toronto**  
**Pre-meal Snacks, Satiety and Food Intake in Children**

## Body Measurements

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<thead>
<tr>
<th>Subject Code:</th>
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<tbody>
<tr>
<td>Date of Birth:</td>
<td></td>
<td>(dd/mm/yy)</td>
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<tr>
<td>Age:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight:</td>
<td>Kg</td>
<td></td>
</tr>
<tr>
<td>Height:</td>
<td>cm</td>
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### SKINFOLDS

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<tr>
<th>Measurement</th>
<th>Visit 1</th>
<th>Visit 2</th>
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</thead>
<tbody>
<tr>
<td>Biceps (mm)</td>
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<td></td>
</tr>
<tr>
<td>Tricep (mm)</td>
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<tr>
<td>Subscapular (mm)</td>
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</table>

### Average Measurement

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<tr>
<th>Measurement</th>
<th>Visit 1</th>
<th>Visit 2</th>
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### BIA

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<tr>
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<tbody>
<tr>
<td>Resistance</td>
<td></td>
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<tr>
<td>Reactance</td>
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<tr>
<td>Body Fat %</td>
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</table>

### Waist Circumference

<table>
<thead>
<tr>
<th>Visit</th>
<th>cm</th>
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<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
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</tbody>
</table>
Appendix 7. Study Day Questionnaires

7.7.1 Feeding Session Cover Sheet

Feeding Session Cover Sheet
Department of Nutritional Sciences, University of Toronto

Milk Mixed Meal Food Intake Control in Children

Subject ID: ___________________________ Session: ________________

Date: ________________________________

Baseline Questionnaire (to be asked by investigator)

1. Have you had the standardized breakfast this morning? YES / NO

2. At what time did you have the standardized breakfast? ________________________________

3. Have you had anything to eat or drink for 10 - 12 hours before breakfast? YES / NO

   If yes, please describe briefly ______________________________________________________

4. Have you had anything to eat or drink after breakfast before arriving here? YES / NO

   If yes, please describe briefly ______________________________________________________

5. Are you taking any medication? YES / NO

   If yes, please describe briefly ______________________________________________________

Comments/Notes:

Investigator: ______________________________
7.7.2 Motivation to Eat Visual Analogue Scale (VAS)

Visual Analogue Scale
Motivation to Eat

Name: ________________________
Date: ________________________

These questions relate to your “motivation to eat” at this time. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very WEAK —————————————————— Very STRONG

2. How hungry do you feel?

NOT Hungry at all —————————————————— As hungry as I have ever felt

3. How full do you feel?

NOT Full at all —————————————————— VERY Full

4. How much food do you think you could eat?

NOTHING at all —————————————————— A LARGE amount

5. How thirsty do you feel?

NOT Thirsty at all —————————————————— As thirsty as I have ever felt
7.7.3 Physical Comfort VAS

Visual Analogue Scale
Physical Comfort

Name: ________________________
Date: ________________________

These questions relate to your “physical comfort” at this time. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

1. How well do you feel?

   NOT well _______________________________    VERY Well
   at all
7.7.4 Beverage Pleasantness VAS

Visual Analogue Scale
Pleasantness

Name: ______________________

Date: ______________________

This question relates to the palatability of the drink you just consumed. Please rate the pleasantness of the drink by placing a small ‘x’ across the horizontal line at the point which best reflects your present feelings.

1. How pleasant have you found the drink?

<table>
<thead>
<tr>
<th>NOT at all</th>
<th>____________________________</th>
<th>VERY pleasant</th>
</tr>
</thead>
<tbody>
<tr>
<td>pleasant</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


7.7.5    Beverage Sweetness VAS

Visual Analogue Scale  
Sweetness

Name: ________________________  
Date: ________________________  

This question relates to the palatability of the drink you just consumed. Please rate the sweetness of the drink by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

1. How sweet have you found the drink?

NOT sweet  ___________________________________________  VERY sweet
at all
7.7.6 Food Pleasantness VAS

Visual Analogue Scale

Pleasantness

Name: ________________________

Date: ________________________

This question relates to the palatability of the food you just consumed. Please rate the pleasantness of the food by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

1. How pleasant have you found the food?

NOT at all ___________________________ VERY pleasant

please
7.7.7 Sick Kids Milk Study Case Report Form

**Milk Study - Case Report Form**

Patient ID: __________

**VISIT 1**

Date of Visit: \( dd/mmm/yy \) Age:

Height: __________ Weight: __________ BMI: ______

Gender: __________

Do you have any allergies?

Food  □ Yes  □ No  If yes please specify-----------------------------------------------

Drugs □ Yes □ No  If yes please specify-----------------------------------------------

**BASELINE QUESTIONS**

1. Have you had the standardized breakfast this morning? YES / NO

2. At what time did you have the standardized breakfast? _____

3. Have you had anything to eat or drink for 10 - 12 hours before breakfast? YES / NO

   If yes, please describe briefly ________________

4. Have you had anything to eat or drink after breakfast before arriving here? YES / NO

   If yes, please describe briefly ________________

5. Are you taking any medication? YES / NO

   If yes, please describe briefly ________________

Comments/Notes:
Investigator: ________________

LABORATORY SAMPLES

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<thead>
<tr>
<th></th>
<th>0 mins</th>
<th>30 mins</th>
<th>60 mins</th>
<th>85 mins</th>
<th>115 mins</th>
<th>145 mins</th>
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<td>Glucose</td>
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<tr>
<td>Insulin</td>
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<td>X</td>
<td></td>
<td></td>
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<td>PYY</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Active Ghrelin</td>
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<td></td>
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<tr>
<td>CCK</td>
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<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

FOOD ADMINISTRATION RECORD

**Snack** (please circle): MILK / JUICE

Start time: _______  Stop time: _______

Completed within 5 minutes (please circle): YES / NO

**Meal**:
Start time: _______  Stop time: _______  Amount Consumed: _______

VISUAL ANALOG SCALE

**Motivation to Eat**
(Measured at 0, 10, 30, 60, 85, 95, 105, 115, 145 minutes)

These questions relate to your “**motivation to eat**” at this time. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

5. How strong is your desire to eat?

Very ___________________________  Very STRONG

WEAK ___________________________________

6. How hungry do you feel?

 NOT ___________________________  As hungry

Hungry ___________________________  as I have

at all ________________________________________  ever felt
7. How full do you feel?

    NOT ________________________________  VERY
    Full
    at all  Full

8. How much food do you think you could eat?

    NOTHING ___________________________  A LARGE
    at all  amount

**Physical Comfort**
(Measured at 0, 10, 30, 60, 85, 95, 105, 115, 145 minutes)
These questions relate to your “**physical comfort**” at this time. Please rate yourself by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

2. How well do you feel?

    NOT well ___________________________  VERY
    at all  Well

**Pleasantness**
(Measured at 5 and 85 minutes)
This question relates to the palatability of the food/drink you just consumed. Please rate the **pleasantness** of the food/drink by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

2. How pleasant have you found the food/drink?

    NOT at all ___________________________  VERY
    pleasant  pleasant

**Sweetness**
(Measured at 5 and 85 minutes)
This question relates to the palatability of the food/drink you just consumed. Please rate the **sweetness** of the food/drink by placing a small “x” across the horizontal line at the point which best reflects your present feelings.

1. How pleasant have you found the food/drink?

    NOT ________________________________  VERY
    sweet  sweet
    at all  at all
Appendix 8. Pizza Meal Records & Blood Collection Records

7.8.1 Experiment 1: Pizza Meal Records

<table>
<thead>
<tr>
<th>Subject ID:</th>
<th>Pizza Preference:</th>
</tr>
</thead>
<tbody>
<tr>
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<table>
<thead>
<tr>
<th>Subject ID:</th>
<th>Pizza Preference:</th>
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## 7.8.2 Experiment 2: Pizza Meal Records

**Subject ID:** _____________  **Pizza Preference:** ___________________________

**Session:** _______  **Treatment:** ______________

**Investigator:** _______________  **Date:** _______________

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**Treatment (g)**
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| 2 |   |       | |

**Water (g)**
| 1 |   |       | |
| 2 |   |       | |

**Session:** _______  **Treatment:** ______________

**Investigator:** _______________  **Date:** _______________

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**Treatment (g)**
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| 2 |   |       | |

**Water (g)**
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| 2 |   |       | |
7.8.3  Experiment 2: Blood Collection Records

**MILK STUDY – Blood Collection Record Sheet # ____**

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Appendix 9. Miscellaneous Forms

7.9.1 Experiment 1: Reminder Forms

Thank you for your participation!

Your next appointment is ______________ at ______ am.

You will be done by _______ at the latest.

Location: 150 College St (College and Queens Park)
FitzGerald Building. Please wait at the brown door facing College St.

Remember to fast for 12 hrs before eating the provided breakfast @ ______ am on the day of the study. Please eat the entire breakfast, but do not eat or drink anything else.

You may bring homework or quiet entertainment to use ONLY when in room 320.

If you feel ill, it is better to re-schedule.

If you need to re-schedule or have any questions please call Ms. Shirley Vien @ 416 978 4153
7.9.2  Experiment 2: Instructions & Map

TESTING at SICKKIDS

If you are lost please call Shirley 416-978-4153
Location: PHYSIOLOGICAL RESEARCH UNIT, ROOM 5500 HILL WING
Time: ______

Directions if parking at SickKids:

- Underground lot on Elm St (south side of the hospital)
- Bring parking ticket with you to testing room
- Go up parking elevators to main floor, walk to the left until you reach the elevators by Shoppers Drug Mart (Black Wing Elevators)
- Take elevator to the 5th floor and follow the map to room 5500 Hill Wing

Directions if parking elsewhere or taking public transit:

- On Saturday and Sunday must enter from Elizabeth St (East side of the hospital)
- Walk straight through the Atrium past Starbucks until you reach the elevators by Shoppers Drug Mart (Black Wing Elevators)
- Take the elevator to the 5th floor and follow the map to room 5500 Hill Wing